# CLINICO IMMUNOLOGICAL PROFILE IN CHRONIC LIVER DISEASES

THESIS

FOR DOCTOR OF MEDICINE

(MEDICINE)


BUNDELKHAND UNIVERSITY
JHANSI (U. P.)

1985

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# C E R T I F I C A T E

This is to certify that the work entitled "CLINICO IMMUNOLOGICAL PROFILE IN CHRONIC LIVER DISEASES", which is being submitted as a thesis for M.D. (Medicine) by DR. SUSHANT KUMAR KHARE, has been carried out under my direct supervision and guidance in the department of medicine. The techniques employed in the thesis were undertaken by the candidate himself and the observations recorded have been periodically checked and verified by me.

Dated: 28/7/, 1984.

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# A C K N O W L E D G E M E N T S

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Dated: 30 July, 1984.

Sushant Khare

( SUSHANT KUMAR KHARE )

# CONTENTS

# INTRODUCTION

# INTRODUCTION

Liver diseases are world wide in distribution and a major health problem in developing countries. Over last ten years the concept of chronic liver diseases have come up incorporating chronic hepatitis at one end and cirrhosis of liver at other end of spectrum.

The liver occupies a central position in the metabolism of human body. Being the main clinical laboratory liver plays an important role in the synthesis of plasma proteins such as albumin, perhaps 80% of globulin and those required in blood clotting mechanism (Miller et al., 1954). The liver also plays an important role in the catabolism of serum proteins (Cohen & Gordon, 1959). Thus it is very much expected to have alterations in electrophoretic pattern of serum proteins in chronic liver diseases. Gray & Barron (1943) first established the clinical interpretation of electrophoretic fractions of serum proteins in hepatic disorders. Subsequently other workers have also reported changes in electrophoretic pattern of serum proteins (Feizi, 1968; Sherlock, 1968; Sunderman, 1968). In chronic liver diseases serum gamma globulin rises two or more times to normal. The

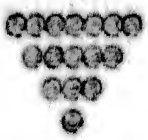
concurrent decrease of serum albumin caused due to diminished synthesis is a measure of liver damage.

The raised levels of immunoglobulins were reported in chronic liver diseases by various workers (Lee,1965 and Doniach et al., 1968). Lo Grippo and associates (1966, 1967 and 1968) quantitated immunoglobulins levels in sera of patients who had viral hepatitis. The raised level of IgG was reported by Feizi (1968) and Lee (1965) in chronic active hepatitis and cirrhosis of liver. High levels of IgM and IgA were reported in primary biliary and alcoholic cirrhosis respectively (Paronetto and Popper, 1964, Mackelvey and Fahey, 1965; Hobbs, 1966).

The clinical and electrophoretic studies might distinguish ascites associated with cirrhosis liver from that due to neoplasm or other conditions, especially when findings in blood and ascitic fluid were compared (Rovelstad et al., 1959). Thus electrophoretic study of ascitic fluid helps in clinical diagnosis. The changes almost similar to that of serum were reported in ascitic fluid by most of the workers (Kay, 1954; Schultz & Heremans, 1966). The immunoglobulins in ascitic fluid were reported in similar ratio as that of plasma although low in absolute concentration.

Chordirker and Tomasi (1964) reported all the immunoglobulins (IgG, IgM & IgA) in ascitic fluid. The IgG & IgA were reported in similar ratios in internal secretions as that of plasma i.e. 5 : 1.

The present work has been designed to study the electrophoretic pattern of serum and ascitic fluid proteins in cases of chronic liver diseases. The immunoglobulins levels were also estimated in serum and ascitic fluid of these patients. The changes were assessed and correlated to ascertain the diagnostic value of these changes.



# REVIEW OF LITERATURE

# REVIEW OF LITERATURE

## SERUM PROTEINS AND THEIR CHANGES

Some of the most important functions of the liver are connected with protein metabolism, such as deamination of amino-acids and the maintenance of normal blood levels of albumin, globulin and fibrinogen. The liver also plays an important role in the synthesis of special proteins such as those required for blood clotting. The protein synthesis is much more rapid in the liver than in any other tissues. For example, in man half life of liver proteins is 2.5 to 10 days, as compared with muscles which is 150 days (Miller & Bale, 1954).

Miller (1954) has shown that infusion of labelled amino acids into isolated perfused dog liver resulted into synthesis of plasma proteins. Similarly the experiments with isolated perfused rat liver and with hepatectomized rat demonstrated that the hepatic parenchymal cells synthesize various protein fractions like albumin, lipoproteins, glycoproteins, ceruloplasmin haptoglobin and prothrombin (Martin & Neuberger, 1957; Gordan & Humphrey 1960).

The liver also plays an important role in the catabolism of serum proteins. Gordan & Coehn and their

associates (1960) have shown that rat liver is responsible for catabolism of 13% albumin, 30% of serum globulin and also serum transferrin.

In the light of major contribution of liver in synthesis and catabolism of proteins, it is not surprising that profound alterations in electrophoretic pattern of serum proteins are observed in liver diseases. Normal person makes about 10.0 g of albumin daily. Hypoalbuminaemic patients with cirrhosis can synthesize only about 4.0 g albumin, 2.0 g of fibrinogen and 1 g transferrin daily (Sherlock, 1972; Rosenoer, 1968).

A rise and fall of plasma proteins concentration may reflect changes not only in hepatic production but also in plasma volume. Total exchangeable protein (Albumin pool) is not depleted in cirrhosis. When ascites is present however, the extra vascular albumin pool is expanded at the expense of the intra vascular one (Wilkinson & Mendenhall, 1963). However, changes are slow to develop and do not immediately reflect acute liver damage. Even complete cessation of albumin production results in only 25% decrease in serum levels after eight days. In patients with continuing cholestasis serum albumin falls. The characteristic change in chronic

liver disease is a fall in serum albumin and rise in serum globulin levels.

In severe prolonged viral hepatitis and in cirrhosis serum albumin level bears a close relationship to the clinical stage and are helpful prognostically and in following treatment. Hyperglobulinaemia is a feature of chronic hepatocellular disease. It reflects a reticuloendothelial reaction to antigens. Extremly high values may characterize chronic active hepatitis and levels falling only with steroid therapy in latter stages of diseases (Sherlock, 1975).

The total plasma proteins average just over 7.0 g% with a range from 6.3 to 7.9 g%. The value for the main constituents of different fractions of serum proteins are given a little different by different workers, but may be taken to be approximately 3.7 to 5.3 g% for albumin, 1.6 to 3.6 g% for globulin and 200 to 400 mg% for fibrinogen, thus giving a albumin and globulin ratio 2.5 :1 to 2 : 1 (Varley, 1980). The above data are obtained by salting out technique. The normal values of different electrophoretic fractions of serum proteins may vary from one laboratory to another depending upon the technique used. However, commonly accepted values for different electrophoretic fractions

of serum proteins as reported by King & Wooton (1956) are as follows :-

- Total serum proteins - 6 to 8 g/100 ml
- Serum Albumin - 3.8 to 5.0 g/100 ml(55-65%)
- Serum Alpha globulins - 0.5 to 1.2 g/100ml ( 4-14%)
- Serum Beta globulin - 0.46 to 1.2 g/100 ml(7-15%)
- Serum Gamma globulin - 0.9 to 1.9 g/100 ml (6-16%)

Martin (1960); Mavens & Williams (1948) and Ricketts & Sterling (1949) reported that the concentration of total serum proteins was not significantly reduced in acute viral hepatitis, but beta globulin may be increased and gamma globulin was found to be with in normal limits. Diminished concentration of Alpha globulins has been co-related with the severity of the viral hepatitis (Dommelen et al, 1959). These changes occur early in the course of disease and may be detected before the appearance of jaundice and usually disappear with in 8 to 12 weeks (Havens,1962; Krugmen and Wander, 1962).

Progressive increase in the concentration of gammaglobulin during follow up after the onset of viral hepatitis may be observed in patients who are undergoing a transition from acute to chronic hepatitis (Ossarmen and Takatuski, 1963). Similarly a marked decrease in

serum albumin and an increase in gamma globulin may as such reflect to a large extent the level of circulating antibody (King & Wooton, 1956)

Chronic persistent hepatitis is a benign disease. Serum globulin levels may be slightly elevated during the first year of disease but return to normal there after (Becker et al., 1970). In patients of chronic active hepatitis marked decrease in total albumin and a greatly increased gamma globulin concentration has been reported by Gelzayd & Krisnar (1967) and Mistillis and Blackburn (1970).

In portal cirrhosis there was a significant decrease in the mean concentration of total serum proteins, serum albumin and alpha globulins, whereas the concentration of beta and gamma globulin was increased markedly (Gray & Barron, 1943; Sunderman et al., 1968). In these patients a profound decrease in serum albumin concentration is attended by a poor prognosis (Post and Patek, 1942). A characteristic feature of the electrophoretic pattern in hepatic cirrhosis is the phenomenon of beta-gamma bridging which means a lack of demarcation between the peaks of beta and gamma globulin (Demeulenere and Weimes, 1961). The increase in the level of globulin is mostly due to increase synthesis by reticulo-endothelial

cells (Cohen, 1963 and Anderson, 1964).

In post necrotic cirrhosis the concentration of gamma globulin is usually greater than in Laennec's cirrhosis and the degree of hypergammaglobulinaemia may be a valuable laboratory aid in the differentiation of these forms of cirrhosis (Gross et al., 1959; Wolff et al., 1958 and Paronetto et al., 1962).

In cryptogenic cirrhosis the serum gamma globulin may rise five to seven times above normal. The hypergammaglobulinaemia of this magnitude is ordinarily not seen in cirrhosis of alcoholics and its presence is therefore of diagnostic value (Bjornboe and Reaschon, 1949 & Zimmerman, 1961).

In primary biliary cirrhosis the hyperbetaglobulinaemia with hypoalbuminaemia was reported by Ahrens and Coworkers, 1949 and Sterling & Ricketts, 1949). In Cholestasis the serum albumin is usually normal untill the terminal cell failure. When serum albumin falls and globulin increases (Sunderman et al., 1968).

In metastatic carcinoma of liver the mean concentration of total serum proteins was found to be reduced significantly. The concentration of alpha-1 globulin was increased and those of beta and gamma globulins were normal (Sunderman and Jared, 1968). The Alpha-1

globulin contains glycoproteins and is low in hepato-cellular diseases, falling in parallel with the serum albumin. An increase accompanies acute febrile illness and malignant disease (Russ et al., 1956). In primary carcinoma of liver in addition to raised alpha-1 globulin, a rise of alpha-2 globulin may also be encountered (Viallet et al., 1962).

SERUM IMMUNOGLOBULINS

The gamma globulins were first recognised and designated as a distinct group of serum proteins by Tiselius (1937). Tiselius and Kabot demonstrated that the antibodies of serum are present in the gamma globulins. An additional knowledge accumulated, the gamma globulin of serum was found to be composed of at least five distinct globulins with antibody activity separable by antigenic analysis (Committee on nomenclature of human immunoglobulin W.H.O. Bull, 1964).

The four classes of immunoglobulins have been identified known as IgG, (γG, 7S, $\gamma_2$ globulin), IgA (γA, $\beta_2$-A globulin), IgM (γM, 18S, γ macroglobulin) and IgD (γD). The immunoglobulins also include other myeloma proteins which are structurally related to antibodies like myeloma proteins and Bence Jones proteins (Fahey, 1965). Recent work on P-K antibody (reagin) indicates that it belongs to a distinct immunoglobulin class

designated as IgE. It is present in very small amount in serum and has a sedimentation rate approximately 8S (Russell & Weiser, 1971).

The IgM molecules are largely intravascular in location (80% in contrast to 40% for IgG) and are catabolized more rapidly (14% of body pool per day in contrast to 3% per day for IgG). These features of IgM antibody are useful characteristics,if antibody is only needed for short time and where continued production of this size is disadvantageous. Recent evidence indicates that IgM antibody synthesis actually shuts off, when IgG antibody synthesis starts (Fahey, 1965). IgG constitutes the largest part about (75%) of the total immunoglobulins concentrations with a serum level of 1.2 g/100 ml . The serum level of IgM is about 0.12 g/100 ml i.e. about 7% of total immunoglobulins. The serum level of IgA is about 0.39 g/100 ml i.e. 21% of total immunoglobulins (Fahey, 1965 b).

Row and Fahey (1965 a+b) described a new immunoglobulin and labelled it as IgD. The serum level of IgD varies very widely but median level of 0.003g/ 100 ml has been accepted.

Hepatic diseases differed in their pattern of

immunoglobulin disorders as suggested by Fahey (1950). Patients with Laennec's cirrhosis typically had markedly increased IgG and IgA but normal IgM levels. The patients with biliary cirrhosis have shown elevated level of IgM with normal IgG and IgA level. In patients with viral hepatitis Hermans found that all immunoglobulins were increased. In hepatoma IgM tended to be reduced.

Lo Grippo and associates (1966 b) have studied 60 mentally retarded children of viral hepatitis aged 1-15 years in different stages of disease. They noticed a normal level of IgG, IgM and IgA in icteric phase of the disease. In group-2 i.e. after one month of disease IgM values were increased above normal. After 4 months of disease the values of both IgG and IgM were above normal. The values of IgG only was found to be elevated after 6 months of disease. The IgM level came down to normal. The level of IgA remained unchanged. This study suggested that primary response to infectious hepatitis is an elevation of IgM and followed by elevated level of IgG, while IgA level was with in normal limits.

Gleichmann and Deicher (1963) also reported similar observations, but in contrast to the Lo Grippo's findings they noted a very little increase in IgG levels in hepatitis sera. They suggested that increase in IgG

level might be a sign of chronic active hepatitis. In patients with chronic active hepatitis the typical immunoglobulin pattern is of very high IgG globulin level with a notable increase in IgM and IgA level (Lee, 1965). This should be compatible with an immunological response to continuing antigenic stimulus. Similar findings were also reported by Deicher et al (1969); Feizi and Maclachlan et al (1965). In cryptogenic cirrhosis the level of IgG, IgA and IgM all were increased as reported by Deicher et al (1969), Feizi (1968) and Maclachlan (1965). The values of IgG were not that pronounced as in chronic active hepatitis.

In patients with alcoholic cirrhosis the IgA level was markedly elevated but this is not certainly specific for alcoholic cirrhosis (Lee, 1965; Deicher et al., 1969 and Feizi, 1968).

The raised levels of IgM were reported in primary biliary cirrhosis by Feizi, (1961) and Paronetto, F., (1964). This rise is usually not found in drug cholestatis or in extra hepatic biliary obstruction and may therefore be helpful in differential diagnosis of these diseases (Hobbs, 1966 and Sherlock, 1970). Paronetto and Popper reported that in primarily biliary cirrhosis the IgM was present in large basophillic cells aggregation of complement and antibody complex around the bile ducts.

ASCITIC FLUID IN CIRRHOSIS OF LIVER

The development of ascites is the commonest major complication of cirrhosis liver and implied a poor prognosis (Ratnoff and Patek,1942; Sherlock,1958). The ascitic fluid has the characteristic of an ultrafiltrate of plasma augmented by varied amount of plasma proteins and thus represents an expansion of extra cellular space. The constituents are in dynamic state of equilibrium with the remainder of the body fluids (Schoenberger et al, 1952).

Specific immunoelectrophoresis and immunoprecipitin techniques have amply confirmed the presence in the ascitic fluid and other extravascular fluid spaces nearly all the different plasma proteins (Sabo et al, 1963). The transport of albumin into ascitic fluid has long been a source of interest for the accumulation and disappearance of this fluid may occur rapdily and not always in relationship to understandable disease processes. Zimman and co-workers (1969) using isotopic procedures have suggested that all the albumin moving into ascitic fluid in patients of cirrhosis could not have been derived from the systemic circulation. A more direct extra vascular route either by direct

lymphatic drainage or by direct loss through the hepatic capsule might be involved. Analysis of ascitic fluid and its paper electrophoresis may help in clinical diagnosis. A strong qualitative resemblance in protein patterns between ascitic fluid and plasma with a tendency for the albumin and alpha-1 globulin fractions to be present in higher concentrations and for the alpha-2, beta and gamma globulins to be present in slightly lower concentrations in ascitic fluid than the plasma has been reported (Schultze and Hermans, 1966).

Almost all the immunoglobulins (IgG, IgM and IgA) were reported in ascitic fluid (Szabo et al, 1963). Chodirker and Tomasi (1963) reported that IgA is the predominant immunoglobulin in sero mucus secretions. Such secretory IgA is found only in external secretions like salive and tracheobronchial secretions while internal secretions like pleural fluid, ascitic fluid, synovial fluid, amniotic fluid and cerebrospinal fluid, IgA is not of secretory type. The IgG and IgA ratio is similar to plasma that is approximately 5:1 in internal secretions.

# MATERIAL AND METHOD

## MATERIAL AND METHODS

The present study was conducted on a total of 50 patients, suffering from chronic hepatitis and cirrhosis of liver, admitted in the hospital at M.L.B. Medical College, Jhansi. Thirty age and sex matchod healthy subjects served as control.

The detailed clinical history with special reference to dietary habits, consumption of alcohol and hepatotoxic drugs, jaundice and blood transfusion as well as the findings of clinical examination were recorded on a predesigned proforma.

In every case the routine examination of the blood and urine, liver function tests including serum bilirubin, thymol turbidity test, serum alkaline phosphatase, S.G.O.T., S.G.P.T. and serum cholesterol estimation were carried out. The ascitic fluid was examined for total protein and cells in 26 cases who had ascites. The diagnosis of the liver disease was confirmed after liver biopsy.

The healthy volunteers who served as control were carefully evaluated for any apparent disease, special emphasis was paid to eliciting any previous history of jaundice, blood transfusion and of hepatomegaly in recent past.

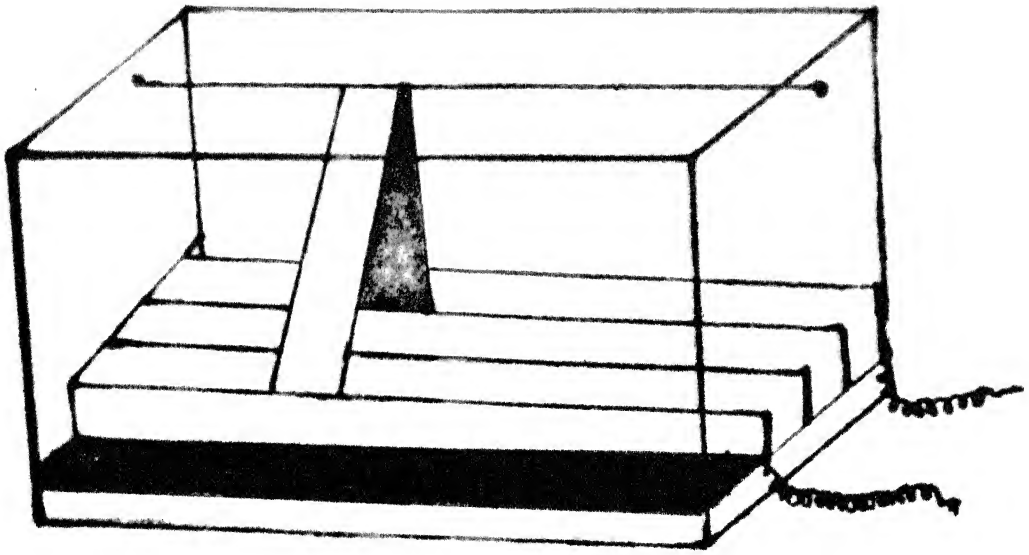
Venous blood (5ml) collected aseptically from patients as well as healthy controls, was allowed to clot in sterlized tubes for half an hour, the serum was separated by centrifugation at 3000 r.p.m. for 10 minutes and stored frozen in sterlized vials (Luxbro) at minus 20°C in a deep freezer.

The ascitic fluid was collected under aseptic condition and stored in storage vials in a deep freezer at minus 20°C.

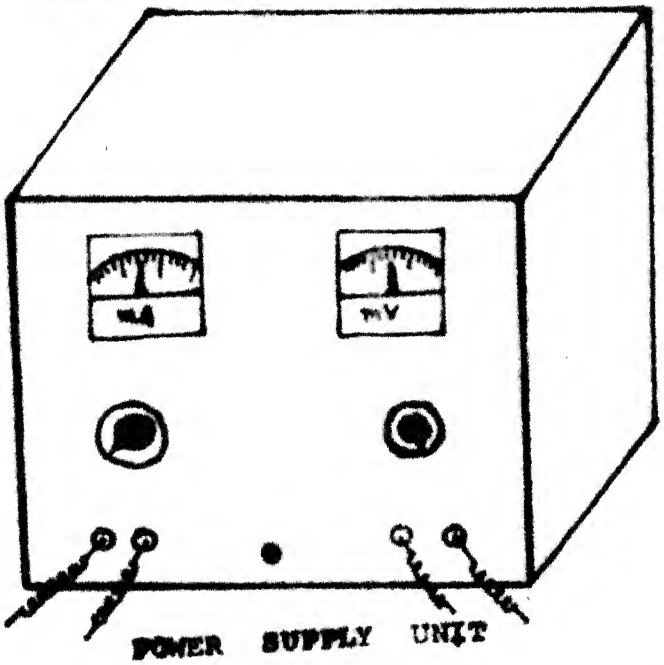
ESTIMATION OF TOTAL SERUM PROTEINS

Total serum proteins were estimated by biuret method as described by Varley (1982). The biuret reagent was prepared by mixing sodium potassium tartrate 4.5 g. copper sulphate 1.5 g. and potassium iodide 5 g. in 0.2 N sodium hydroxide to make one litre of solution.

Four ml of this biuret reagent was mixed with 1 ml of 1:10 diluted serum and incubated at 37°C for 30 minutes in a water bath. The optical density of the colour thus produced was measured calorimetrically at 540 nm. The protein concentration was calculated by using a standard protein curve plotted by using the optical density against the known protein concentration.



ELECTROPHORETIC CHAMBER




POWER SUPPLY UNIT

ECTROPHORETIC APPARATUS USED FOR DETERMINATION OF
RIOUS PROTEIN FRACTIONATIONS IN SERUM & ASCITIC FLUID.

PAPER ELECTROPHORESIS FOR SERUM PROTEINS

Various fractions of the serum proteins were estimated by paper electrophoresis in a vertical electrophoresis tank using 45 x 2.5 cm strips of whatman filter paper No. 3 and barbitone buffer (pH 8.6). The buffer contained 3.12 g of diethyl barbituric acid and 17.1 g of sodium diethyl barbiturate per litre.

On each strip 10 micro litre of serum was applied gently using a cover slip, so that it was evenly spread across the strip and direct current of 1 milli amp./strip was applied for a period of 16 to 18 hours, using a constant power supply unit. After the electrophoresis run the strips were removed, dried at room temperature and fixed in hot air oven at 110°C for 10 minutes. The strips were stained over night in bromophenol blue. This staining solution was prepared by dissolving 100 mg of bromophenol blue in 25 ml of 95% ethenol and 50 g of zinc sulphate in 5% acetic acid was added to it and make upto one litre by 5% acetic acid. The strips were cleared by washing thrice in 5% acetic acid solution, 0.3% sodium acetate dissolved in 5% acetic acid was used as fixative. The strips were dried and stored for quantitative assessment.

PAPER ELECTROPHORESIS OF ASCITIC FLUID

Before subjecting the ascitic fluid to paper electrophoresis the fluid was concentrated by incubating in water bath at 45°C for 8 to 10 hours. Twenty micro litres of ascitic fluid was applied on a strip and subjected to electrophoresis in similar way to that of serum.

ESTIMATION OF VARIOUS FRACTIONS OF PROTEIN IN SERUM AND ASCITIC FLUID

Various fractions of proteins in the serum and ascitic fluid were quantitated using an optical densitometer. The strips were soaked in liquid paraffin and the densitometer readings of various protein fractions were recorded on a graph paper. Thearea occupied by various fractions indicated their proportion in a particular sample. Thus the concentration of various fractions of proteins in the serum as well as ascitic fluid was calculated in terms of percentage as well as in g%.

QUANTITATIVE ESTIMATION OF IMMUNOGLOBULINS IN SERUM

Single radial immuno diffusion(R.I.D.) technique described and developed by Mancini et al (1965) was used. The principal of this method "antigen was deposited at a single point in a small cylindrical well on a thin layer of gel containing a uniform concentration of antibody.

diffuses in to the gel, antigen antibody reaction occurs and a disk shaped immuno precipitate ring is formed. A quantitative relationship exists between the amount of antigen applied to the well and size of resulting precipitin ring. This principle further implied that the area of given precipitin ring after a period of growth due to diffusion of antigen from the well will reach to maximum size and after which no further development will take place and the terminal size 'd' of precipitin ring and square of its diamter '$d^2$' value is linearly proportional to the amount of antigen applied to the well and reciprocal antibody concentration into the gel.

PREPARATION OF AGAR

To the 100 ml of barbitone buffer (pH 8.6) was added 2 g of agrose and mixed by boiling on a water bath untill completely dissolved forming a clear gel.

PREPARATION OF AGAR ANTIGEN MIXTURE

To the 4 ml of molten buffered agrose at 48°C equal amount of 1:10 diluted corresponding antiglobulin serum (Anti IgG, Anti IgM or Anti IgA) prewarmed to 48°C was added and quickly poured on a prewarmed (48°C) 90 mm dia sterlized petridish so as to form a gel of

uniform thickness. After setting of the agrose, wells were punched out using a predesigned card board template, so that the centre of each well was 1 cm apart from one another.

CHARGING OF WELLS

Each well was charged with 5 µl serum using micro capillaries, care was taken to avoid over filling of the wells. For the estimation of immunoglobulin IgG, the sera were diluted to 1:10, where as for the estimation of immunoglobulin IgM undiluted sera were used and for immunoglobulin IgA estimation, 1:2 diluted sera were used.

The plates were incubated at $4^{\circ}C$ for 48 hours in moist environment, diamter of each precipitin ring was measured using a tripartigen scale, under oblique illumination.

ESTIMATION OF IMMUNOGLOBULINS

The immunoglobulins were estimated using a standard curve. Which was prepared by charging the well with serial dilution (100%, 50%, 25% and 12.5 %) of standard sera and the $d^2$ of precipitin rings thus obtained were plotted against the known concentration of immunoglobulin in the standard reference sera. Various antiglobulin antisera and standard reference serum used

in this study were obtained from M/S Hoechst Pvt. Ltd.(Bombay).

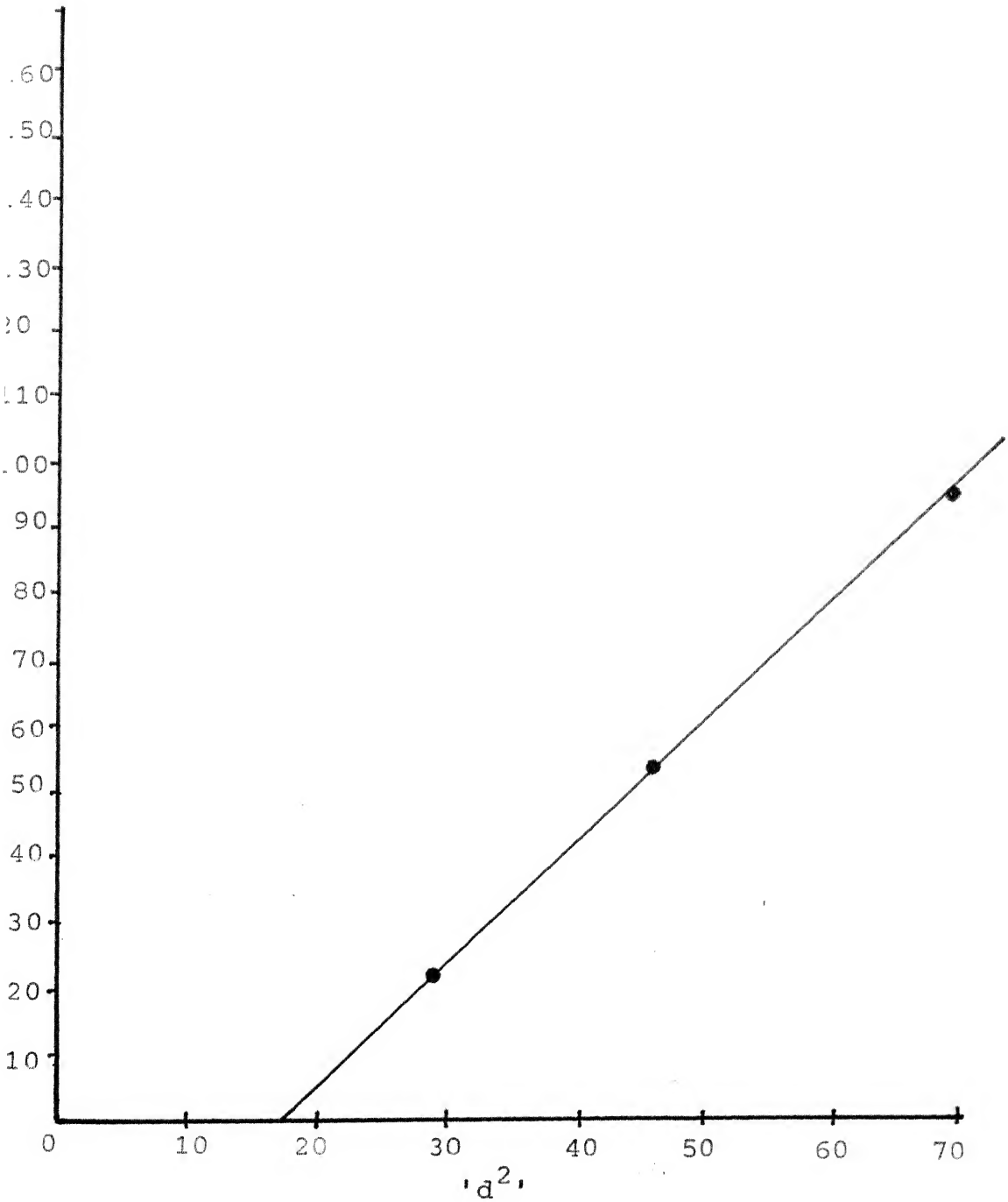
STAINING OF THE PRECIPITIN RINGS

The excess of unprecipitated protein was washed off after several washings (5-6) with sterilised normal saline over a period of 3 days. The plates were covered by filter paper and left at room temperature to dry and were stained using Amidoschwarz 10B for ten minutes. The excess of stain was removed and the precipitin rings were differentiated after several washing with 2% acetic acid.

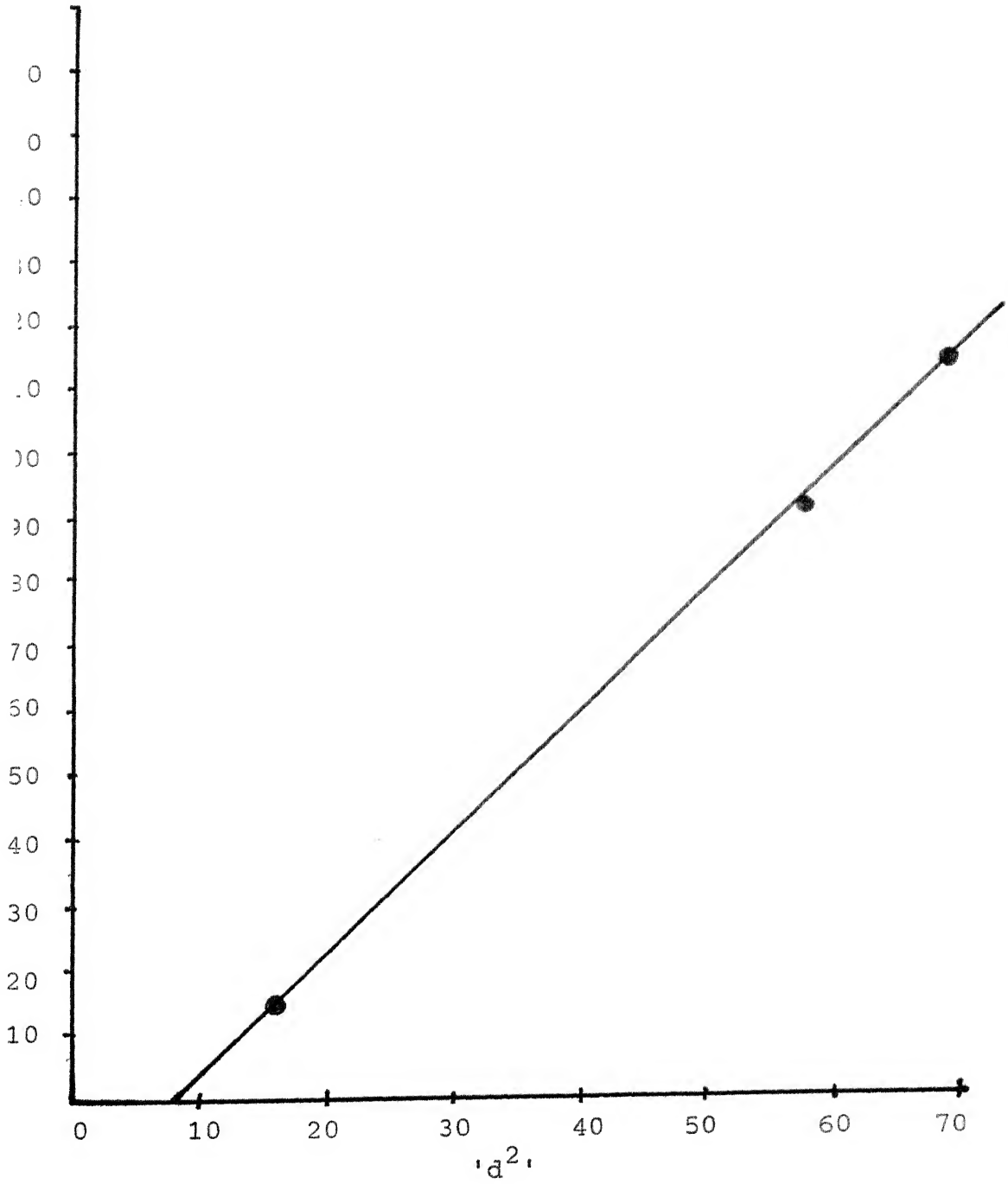
QUANTITATIVE ESTIMATION OF IMMUNOGLOBULINS IN ASCITIC FLUID

Undiluted Ascitic Fluid was used for the estimation of IgG, IgA and IgM immunoglobulins. The terminal size of rings and results were recorded in the similar manner as described for the estimation of immunoglobulins in the serum.

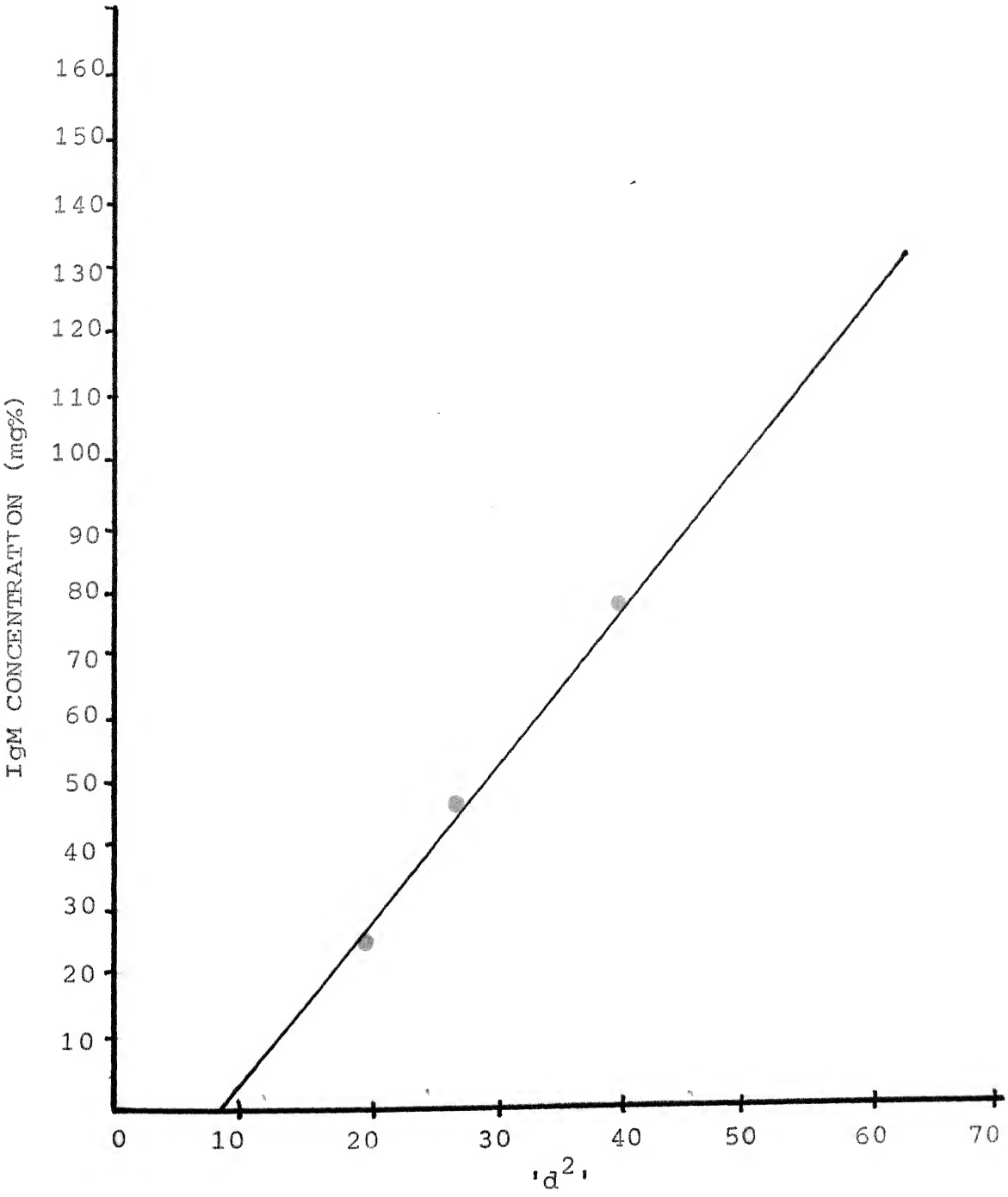
STANDARD CURVE FOR ESTIMATION OF IMMUNOGLOBULIN IgG



STANDARD CURVE FOR ESTIMATION OF IMMUNOGLOBULIN IgA



STANDARD CURVE FOR ESTIMATION OF IMMUNOGLOBULIN IgM



# OBSERVATION

# O B S E R V A T I O N S

The present study was carried out at M.L.B. Medical College Jhansi, in the department of Medicine and Pathology during the period of August, 1983 to April, 1984. The study group consisted of 50 cases of various types of chronic liver diseases viz. cirrhosis of liver, chronic persistent hepatitis and chronic active hepatitis. The number of cirrhosis cases were 38 (76%) while 9 (18%) and 3 (6%) were of chronic persistent hepatitis and chronic active hepatitis respectively (Table I). Thirty healthy individuals were taken to find out normal levels of investigatory parameter in our laboratory. This group included blood donors, doctors, staff nurses, medical students of M.L.B. Medical College Jhansi.

Table I

Distribution of cases of chronic liver diseases.

| Group | No. of cases | Percentage |
|---|---|---|
| Cirrhosis of liver | 38 | 76 |
| Chronic persistent hepatitis | 9 | 18 |
| Chronic active hepatitis | 3 | 6 |
| Total | 50 | 100 |

The age and sex distribution of healthy controls were shown in table II. There were 21 males and 9 females. The majority of male cases (47.62%) were in the age group of 20-30 years with the mean age of 36.05 years. The majority of females (55.6%) were in the age group of 20-30 years with mean age of 25.00 years.

Table II

Age and sex distribution of healthy controls.

| Age group | Male | | Female | | Total | |
|---|---|---|---|---|---|---|
| | No. of cases | Percentage | No. of cases | Percentage | No. of cases | Percentage |
| 20 - 30 | 10 | 47.62 | 5 | 55.6 | 15 | 50 |
| 30 - 40 | 6 | 28.57 | 3 | 33.3 | 9 | 30 |
| 40 - 50 | 5 | 23.81 | 1 | 11.1 | 6 | 20 |
| Total | 21 | 100.00 | 9 | 100.00 | 30 | 100 |

The age and sex distribution of study group is shown in table III. Out of 38 cases of cirrhosis liver, 18 were males and 20 females. Most of the cases were in the age group of 40-60 years (68.42%). There were 5 males and 4 females out of 9 cases of chronic persistent hepatitis. The majority of cases were in the age group of 40-50 years (44.4%). The chronic active hepatitis group consisted of two males and one female. The majority of patients were in the age group of

Table III

Age and sex distribution of study group.

| Disease group | Age group 20-30 | 30-40 | 40-50 | 50-60 | Total | Percentage |
|---|---|---|---|---|---|---|
| Male | 1 | 3 | 4 | 10 | 18 | 36 |
| Cirrhosis | | | | | | |
| Female | 3 | 5 | 7 | 5 | 20 | 40 |
| Male | 2 | - | 2 | 1 | 5 | 10 |
| Chronic persistent hepatitis | | | | | | |
| Female | - | 1 | 2 | 1 | 4 | 8 |
| Male | 2 | - | - | - | 2 | 4 |
| Chronic active hepatitis | | | | | | |
| Female | - | - | - | 1 | 1 | 2 |
| Total | | | | | 50 | 100 |

SERUM PROTEINS IN HEALTHY CONTROLS

The mean concentration of total serum proteins in 30 healthy controls was 6.88 ± 0.62 g% with a range of 5.6 to 8.0 g%. The values for various electrophoretic fractions are shown in table IV. The serum albumin level was 2.86 ± 0.56 g%. The values for alpha-1 and alpha-2 globulins were 0.60 ± 0.47 g% and 0.66 ± 0.26 g% respectively. The beta and gamma globulins levels were 1.05 ± 0.55 g% and 1.71 ± 0.50 g% respectively.

Table IV

Distribution of total serum proteins and their electrophoretic fractions in healthy controls.

(n = 30)

| Total serum proteins g% (Mean±S.D.) | Electrophoretic fractions in g% (Mean±S.D.) | | | | |
|---|---|---|---|---|---|
| | Albumin | Globulins | | | |
| | | Alpha-1 | Alpha-2 | Beta | Gamma |
| 6.88 ±0.62 | 2.86 ±0.56 | 0.60 ±0.47 | 0.66 ±0.26 | 1.05 ±0.55 | 1.71 ±0.50 |

SERUM IMMUNOGLOBULINS IN HEALTHY SUBJECTS

The mean concentration for various immunoglobulins in healthy controls are shown in table V. The immunoglobulin IgG concentration was 1062 ± 243.41 mg% with a range of 700 to 1500 mg%. The IgM and IgA levels were 98.03 ± 43.53 mg% and 227 ± 60.73 mg% with the range of 40 to 150 mg% and 168 to 372 mg% respectively.

Table V

Showing mean concentration of immunoglobulins in healthy subjects. (n = 30).

| Immunoglobulins | Mean ± S.D. (mg %) |
|---|---|
| IgG | 1062 ± 243.41 |
| IgM | 98.03 ± 43.53 |
| IgA | 227 ± 60.73 |

CIRRHOSIS LIVER

This group consisted of 38 cases (18 males and 20 females). A total of 26 cases were having ascites.

The two patients were having clinical jaundice. The mean value for serum bilirubin was 1.15 mg%. The mean concentration for total serum proteins was 6.03±0.94 with a range of 3.0 to 7.7 g%. The total serum proteins was significantly low in comparison to control subjects ($P < 0.001$). The mean albumin level was 1.84±0.55 g%, when compared with control group was found to be significantly lower ($P < 0.001$). The mean concentration of alpha-1 and alpha-2 globulins were 0.63±0.32 g% and 0.68±0.36 g% respectively. These values were found to be higher in comparison to control group ($P > 0.05$). The mean concentration of beta globulin was 0.79±0.53 g% when compared with control group was found to be lower but not significant statistically ($P > 0.05$). The gamma globulin level was 2.08±0.63 g% and found to be significantly higher ($P < 0.01$) in comparison to control group (Table VI).

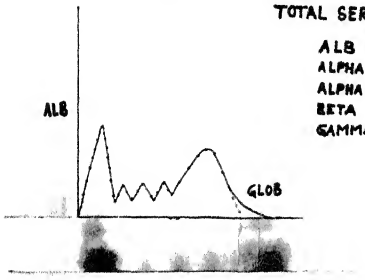
Table VI

Distribution of total serum proteins and their electrophoretic fractions in cirrhosis liver(n=38).

| Serum proteins & its electrophoretic fractions. | Mean Concentration (g%) (Mean±S.D.) | Statistical significance (D.F.=66) t value | P value |
|---|---|---|---|
| Total serum proteins | 6.03±0.94 | 4.44 | <0.001 |
| Albumin | 1.84±0.55 | 3.918 | <0.001 |
| Alpha-1 globulin | 0.63±0.32 | 1.25 | >0.05 |
| Alpha-2 globulin | 0.68±0.36 | 0.28 | >0.05 |
| Beta globulin | 0.79±0.53 | 1.96 | >0.05 |
| Gamma globulin | 2.08±0.63 | 2.57 | <0.01 |

# ELECTROPHORETIC PATTERN IN CIRRHOSIS LIVER

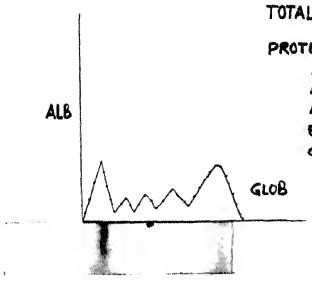
## I In SERUM CASE No-41




TOTAL SERUM PROTEINS - 5·48%

| | |
|---|---|
| ALB | - 29·38% |
| ALPHA-1 | - 10·88% |
| ALPHA-2 | - 14·62% |
| BETA | - 9·34% |
| GAMMA | - 35·19% |

## II In ASCITIC FLUID




TOTAL ASCITIC FLUID - 1·5·9%

PROTEINS

| | |
|---|---|
| ALB | - 21·2% |
| ALPHA-1 | - 13·3% |
| ALPHA-2 | - 16·7% |
| BETA | - 22·2% |
| GAMMA | - 26·5% |

The immunoglobulins levels in cirrhosis group are shown in table VII. The IgG level in cirrhosis of liver was 1165±135.96 with a range of 750 to 1500 mg%. This was found to be significantly higher ($P < 0.05$) in comparison to control group. The IgM level was 89.03±40.26 mg% with a range of 20 to 200 mg%. This value was almost identical with control group and the difference was statistically insignificant ($P > 0.1$). The IgA level was 302.84±82.20 mg% with a range of 126 to 400 mg%. This was significantly higher ($P < 0.001$) in comparison to control group.

Table VII

Distribution of immunoglobulins in patients of cirrhosis liver (n = 38).

| Immunoglobulins | Mean concentration (mg%) (Mean ± S.D.) | Statistical significance (d.f.= 66) t value | P value |
|---|---|---|---|
| IgG | 1165.00±135.96 | 2.30 | $<0.05$ |
| IgM | 89.03± 40.26 | 0.88 | $>0.1$ |
| IgA | 302.84± 82.20 | 4.24 | $<0.001$ |

ASCITIC FLUID IN CIRRHOSIS LIVER

There were 26 cases of cirrhosis liver who were having ascites. The mean value for total ascitic fluid protein was 1.78±0.61 g% with a range of 0.7 to 5.0 g%.

The three patients were having ascitic fluid protein more than 2.5 g% and in remaining cases it was less than 2.5 g%. The proportion of various electrophoretic fractions in ascitic fluid is shown in table VIII. The mean albumin level was 0.527±0.32 g%. The value for alpha-1 and alpha-2 globulins were 0.248±0.020 g% and 0.224±0.05g%. The beta and gamma globulins levels were 0.257±0.19 g% and 0.513±0.30 g% respectively. The percentage of albumin in ascitic fluid was 29.6 % while in serum 30.5 % of the total proteins respectively. (Table IX). The percentage of alpha-1 globulin in ascitic fluid was 13.9 % while in serum of patients of cirrhosis, it was 10.5 % of total proteins. Thus higher proportion of alpha-1 globulin was noted in ascitic fluid. The beta and gamma globulins were 14.5 % and 28.8% in ascitic fluid proteins. The percentage of alpha-2 globulin was 12.6 % in ascitic fluid as compared to 11.4 % in serum. The gamma globulin concentration was lower in ascitic fluid as compared to serum 28.8% and 34.5 % respectively (Table IX).

**Table VIII**

**Distribution of ascitic fluid proteins and their electrophoretic fractions in patients of cirrhosis liver (n = 26)**

| Total Ascitic fluid proteins g% (Mean ± S.D.) | Electrophoretic fraction g% (Mean ± S.D.) | | | | |
|---|---|---|---|---|---|
| | Albumin | Globulins | | | |
| | | Alpha-1 | Alpha-2 | Beta | Gamma |
| 1.78 ±0.61 | 0.527 ±0.32 | 0.248 ±0.20 | 0.224 ±0.05 | 0.257 ±0.19 | 0.513 ±0.30 |

TABLE IX

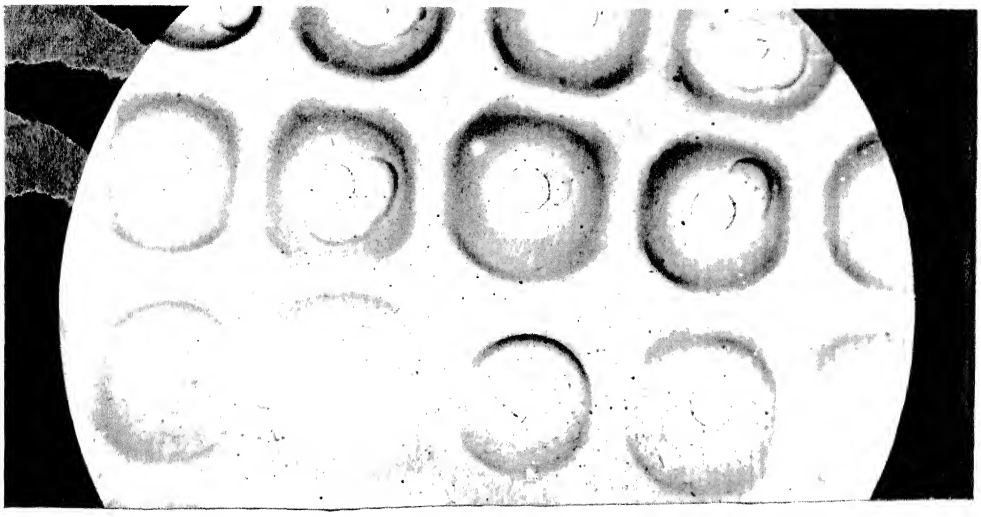
Showing percentage distribution of various electrophoretic fractions in ascitic fluid protein and in serum proteins of patients of cirrhosis liver.

| Cirrhosis liver | Percentage of electrophoretic fractions | | | | |
|---|---|---|---|---|---|
| | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
| Ascitic fluid proteins | 29.6 | 13.9 | 12.6 | 14.5 | 29.8 |
| Serum proteins | 30.5 | 10.5 | 11.4 | 13.1 | 34.5 |

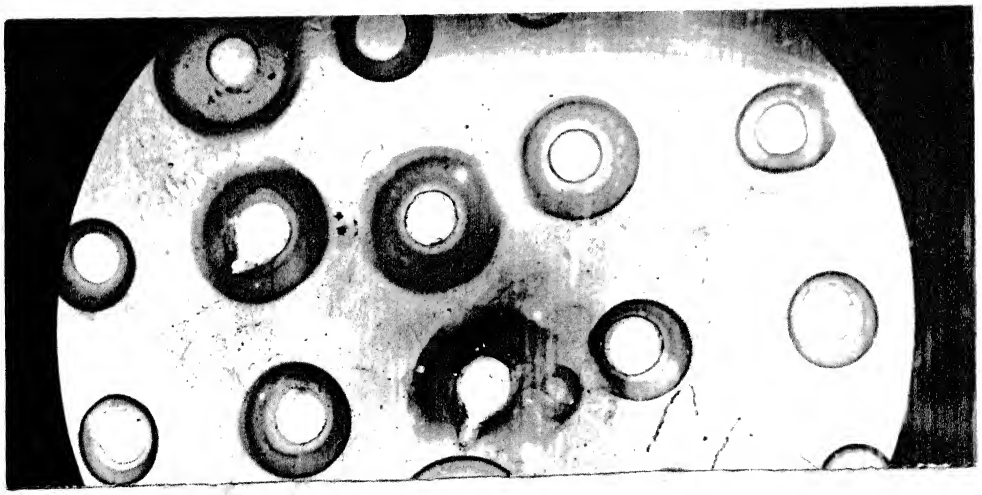
The immunoglobulin concentration in ascitic fluid is shown in table X. The IgG level was 130±24.02 mg%. The IgM and IgA levels were 47.58±21.24 mg% and 97.57 ± 26.08 mg% respectively. The proportion of various immunoglobulins in ascitic fluid and serum of patients of cirrhosis liver is shown in table XI. The IgG was predominant immunoglobulin in serum 74.8% while the percentage of IgG in ascitic fluid was 47.30%. The proportion IgA in ascitic fluid was higher 35.5 % as compared to 19.49 % in serum. The percentate of IgM in ascitic fluid was 17.20 % and in serum 5.71 %. Thus IgG and IgA ratio in serum and ascitic fluid were 3.9 : 1 and 1.4 : 1 respectively.

SINGLE RADIAL IMMUNODIFFUSION TECHNIQUE FOR ESTIMATION OF IMMUNOGLOBULINS.

I. SERUM IMMUNOGLOBULIN IgG IN CIRRHOSIS LIVER.



II. IMMUNOGLOBULIN IgM IN ASCITIC FLUID (CIRRHOSIS LIVER).



## Table X

Showing distribution of immunoglobulins in ascitic fluid in patients of cirrhosis liver.

(n = 26 )

| Immunoglobulins | Immunoglobulins in mg% (Mean ± S.D.) |
|---|---|
| IgG | 130 ± 24.02 |
| IgA | 97.57 ± 26.08 |
| IgM | 47.58 ± 21.24 |

## Table XI

Showing percentage distribution of immunoglobulins in ascitic fluid and serum of patients of cirrhosis liver.

| Cirrhosis liver | Immunoglobulins distribution in percentage | | |
|---|---|---|---|
| | IgG | IgM | IgA |
| Ascitic fluid | 47.30 | 17.20 | 35.5 |
| Serum | 74.80 | 5.71 | 19.49 |

CHRONIC PERSISTENT HEPATITIS

The total of 9 cases were studied in this group. The age and sex distribution is shown in table III. The five cases were males and remaining four were females. The mean value for serum proteins was 6.15±0.79 g% with a range of 5 to 6.8 g%. The values for different electrophoretic fractions are shown in table XII. The mean concentration of serum albumin was 1.69±0.27 g%. The total serum proteins and serum albumin were lower in comparison to control group. Which was statistically

significant with P values $<0.01$ and $<0.001$ respectively. The mean concentration of alpha-1 and alpha-2 globulins were $0.94\pm0.34$ and $0.87\pm0.35$ g% respectively. These values were higher in comparison to control group but not statistically significant ($P > 0.5$). The beta and gamma globulins were $0.85\pm0.40$ g% and $1.80\pm0.33$ g% respectively. The beta globulin level was found to be low in comparison to control, but the difference was not statistically significant ($P > 0.1$). The gamma globulin level was higher in comparison to control group, which was statistically insignificant ($P > 0.5$) Table XII.

Table XII

Distribution of total serum proteins and their electrophoretic fractions in chronic persistent hepatitis (n = 9).

| Serum proteins and its electrophoretic fractions. | Mean Concentration in g% (Mean ± S.D.) | Statistical significance (d.f. = 37) | |
|---|---|---|---|
| | | t value | P value |
| Total serum proteins | 6.15 ± 0.79 | 3.00 | $<0.01$ |
| Albumin | 1.69 ± 0.27 | 3.55 | $<0.001$ |
| Alpha-1 globulin | 0.94 ± 0.34 | 0.297 | $>0.5$ |
| Alpha-2 globulin | 0.87 ± 0.35 | 0.094 | $>0.5$ |
| Beta globulin | 0.85 ± 0.40 | 1.06 | $>0.1$ |
| Gamma globulin | 1.80 ± 0.33 | 0.509 | $>0.5$ |

The table XIII shows the distribution of immunoglobulins in patients of chronic persistent hepatitis. The IgG level was 1104 ± 163.17 mg%. Thus higher level was noted in comparison to control group, but statistically insignificant (P 7 0.5). The IgM level was almost identical to control group and the difference was not significant (P 7 0.5). The raised level of IgA was noted 301.11 ± 52.25 mg% and was found to be statistically significant (P < 0.05).

Table XIII

Immunoglobulins levels in patients of chronic persistent hepatitis (n = 9).

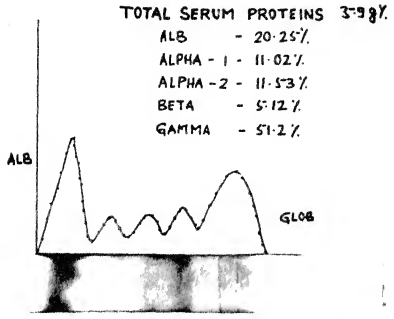
| Immunoglobulins | Mean Concentration in mg% (Mean ± S.D.) | Statistical significance (d.f. = 37) | |
|---|---|---|---|
| | | t value | P value |
| IgG | 1104.00±163.17 | 0.48 | 7 0.5 |
| IgM | 94.00± 4.64 | 0.302 | 7 0.5 |
| IgA | 301.11± 52.25 | 2.5 | < 0.05 |

CHRONIC ACTIVE HEPATITIS

The total of 3 cases were studied in this group two males and one female. The mean age of patients was 30 years. The mean value for total serum proteins was 5.0 ± 1.28 g% with a range of 3.9 to 6.2 g%. This was found to be significantly low in comparison to control

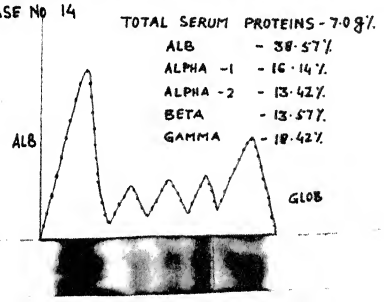
## ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN CHRONIC ACTIVE HEPATITIS

CASE No 23




## ELECTROPHORETIC PATTERN OF SERUM PROTEINS IN HEALTHY CONTROLS

CASE No 14



group (P $<$0.001). The mean concentration for albumin and alpha-1 were 1.39±0.71 g% and 0.61±0.11 g% respectively. The lower level of albumin was noted in comparison to control group and was found to be statistically significant (P $<$0.01). The alpha-2 and beta globulins levels were 0.53±0.18 g% and 0.64±0.48 g% respectively. The mean concentration of alpha-1, alpha-2 and beta globulins were lower in comparison to control group, but statistically was not significant with P values $>$0.1, $>$0.02 and $>$0.1 respectively. The gamma globulin level was raised 1.79±0.46 g% but difference was not found to be statistically significant (P $>$0.5).

Table XIV

Distribution of total serum proteins and its electrophoretic fractions in patients of chronic active hepatitis (n = 3).

| Serum proteins and its electrophoretic fractions. | Mean Concentration g% (Mean ± S.D.) | Statistical significance (d.f. = 31) | |
|---|---|---|---|
| | | t value | P value |
| Total serum proteins | 5.00 ± 1.28 | 5.0 | $<$ 0.001 |
| Albumin | 1.39 ± 0.71 | 2.9 | $<$ 0.01 |
| Alpha-1 globulin | 0.61 ± 0.11 | 1.12 | $>$ 0.1 |
| Alpha-2 globulin | 0.53 ± 0.18 | 2.43 | $>$ 0.02 |
| Beta globulin | 0.64 ± 0.48 | 1.35 | $>$ 0.1 |
| Gamma globulin | 1.79 ± 0.46 | 0.296 | $>$ 0.5 |

The mean concentrations for various immunoglobulins are shown in table XV. The immunoglobulin IgG level was 1426.60 ± 64.82 mg% and was found to be significantly higher in comparison to control group ($P < 0.01$). The mean concentration of IgA and IgM were 283.37 ± 102.70 mg% and 104.0 ± 4.07 mg% respectively. Thus concentration of IgA and IgM were raised in comparison to control group but was insignificant with P value $> 0.1$ and $> 0.5$ respectively.

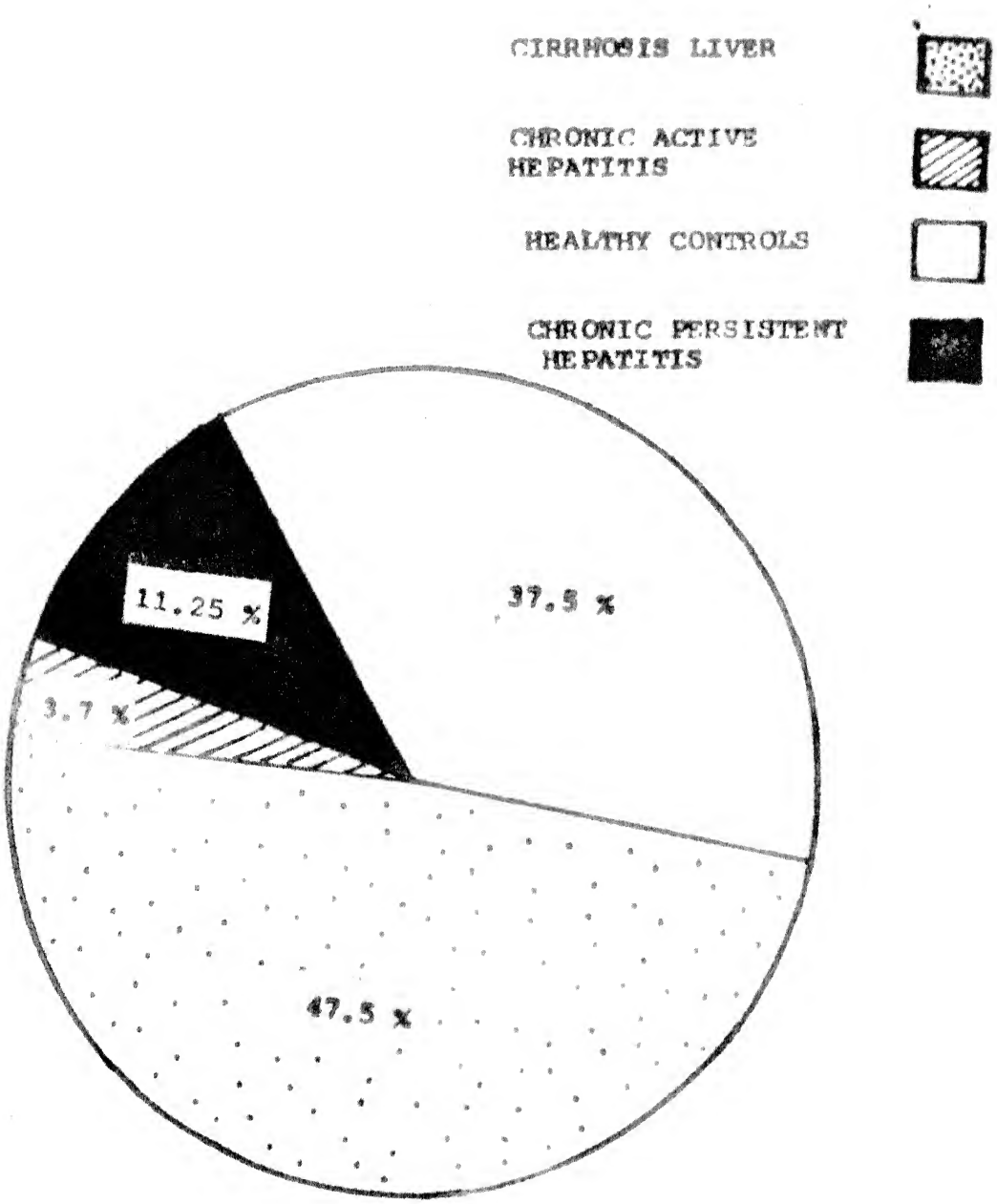
Table XV

Immunoglobulins level in patients of chronic active hepatitis (n = 3).

| Immunoglobulins | Mean Concentration in mg% (Mean ± S.D.) | Statistical significance (d.f. = 31) | |
|---|---|---|---|
| | | t value | P value |
| IgG | 1426.60 ± 64.82 | 2.75 | $< 0.01$ |
| IgA | 283.37 ± 102.70 | 1.55 | $> 0.1$ |
| IgM | 104.00 ± 4.07 | 0.285 | $> 0.5$ |

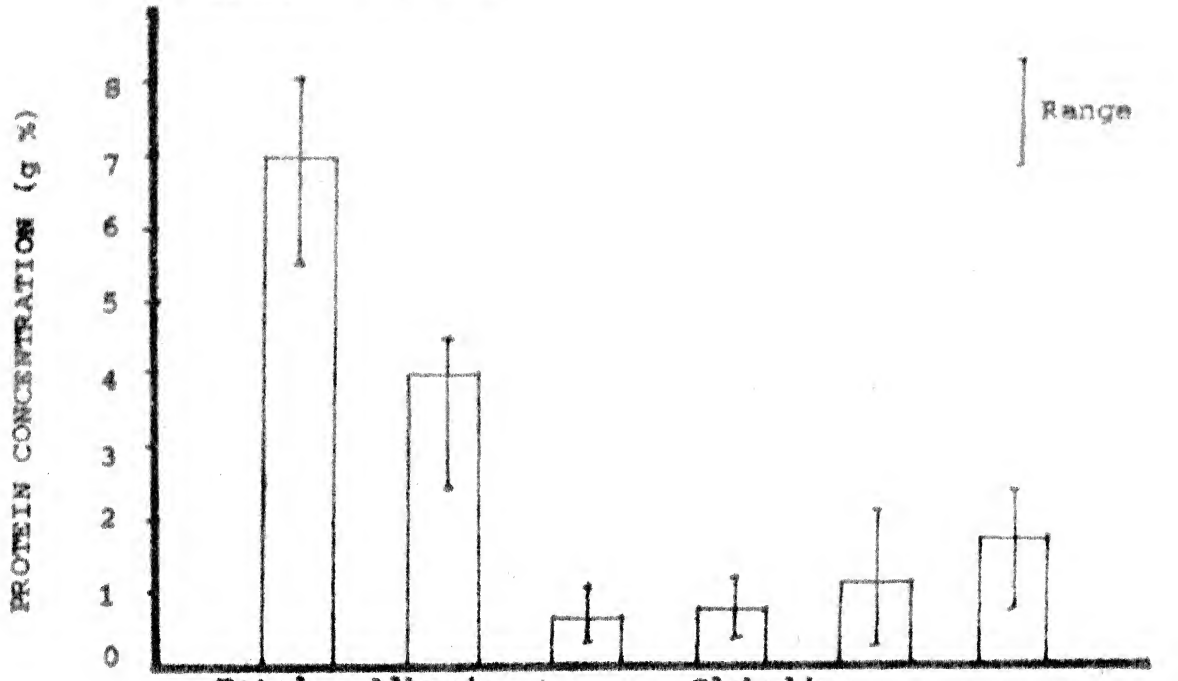
DISTRIBUTION OF CASES

(CONTROLS AND STUDY GROUP)



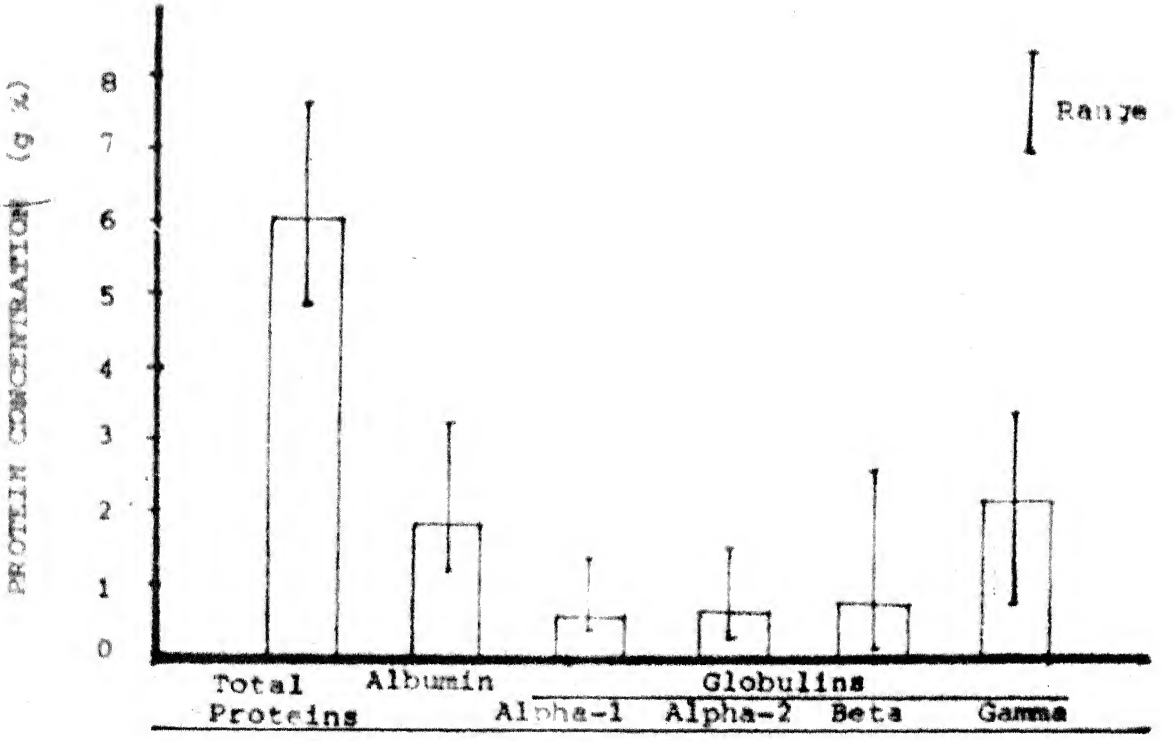
SERUM ELECTROPHORETIC FRACTIONATIONS

1. HEALTHY CONTROLS



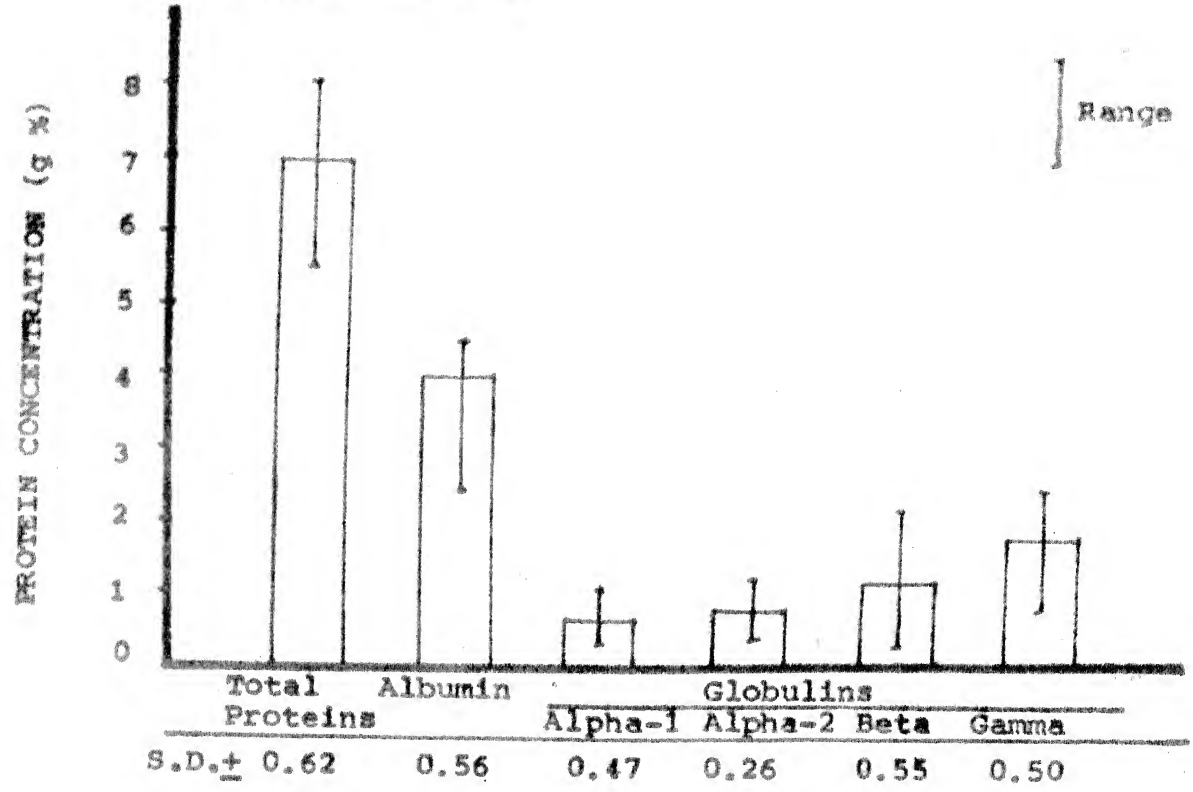

| | Total Proteins | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
|---|---|---|---|---|---|---|
| S.D.± | 0.62 | 0.56 | 0.47 | 0.26 | 0.55 | 0.50 |

2. CIRRHOSIS LIVER



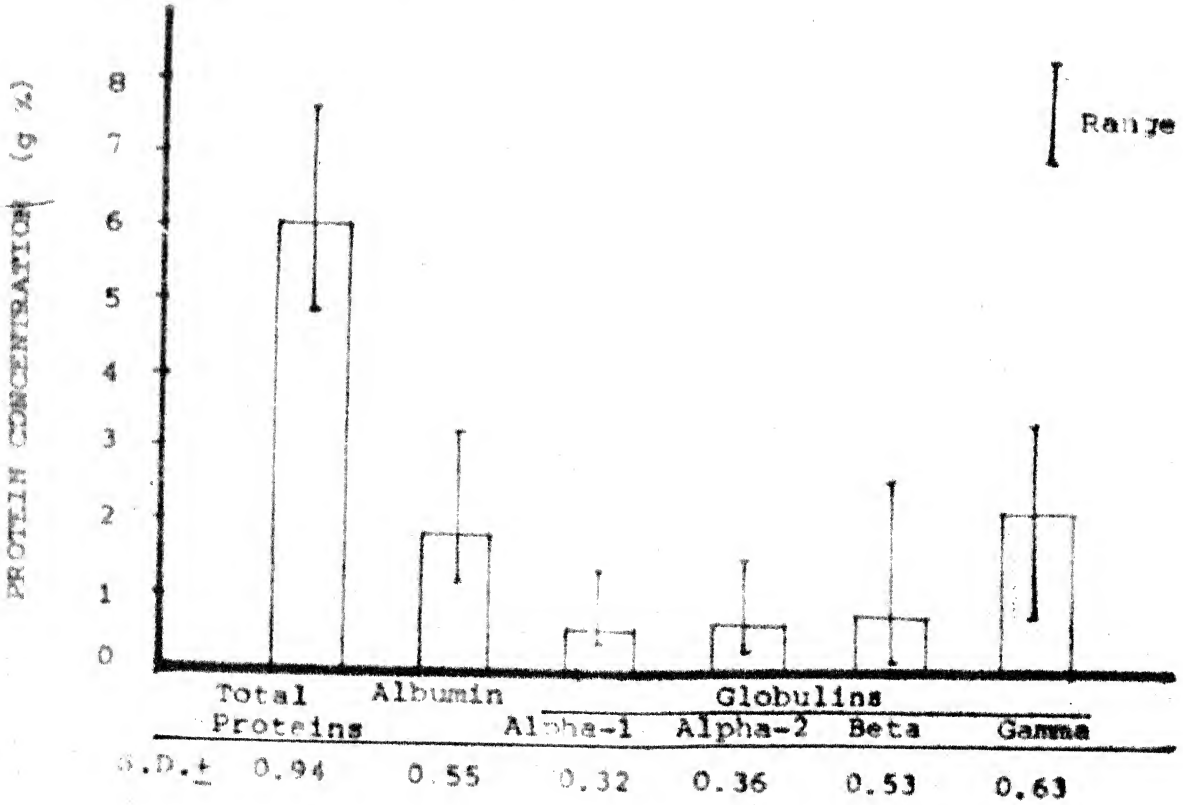
SERUM ELECTROPHORETIC FRACTIONATIONS

1. HEALTHY CONTROLS




| | Total Proteins | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
|---|---|---|---|---|---|---|
| S.D.± | 0.62 | 0.56 | 0.47 | 0.26 | 0.55 | 0.50 |

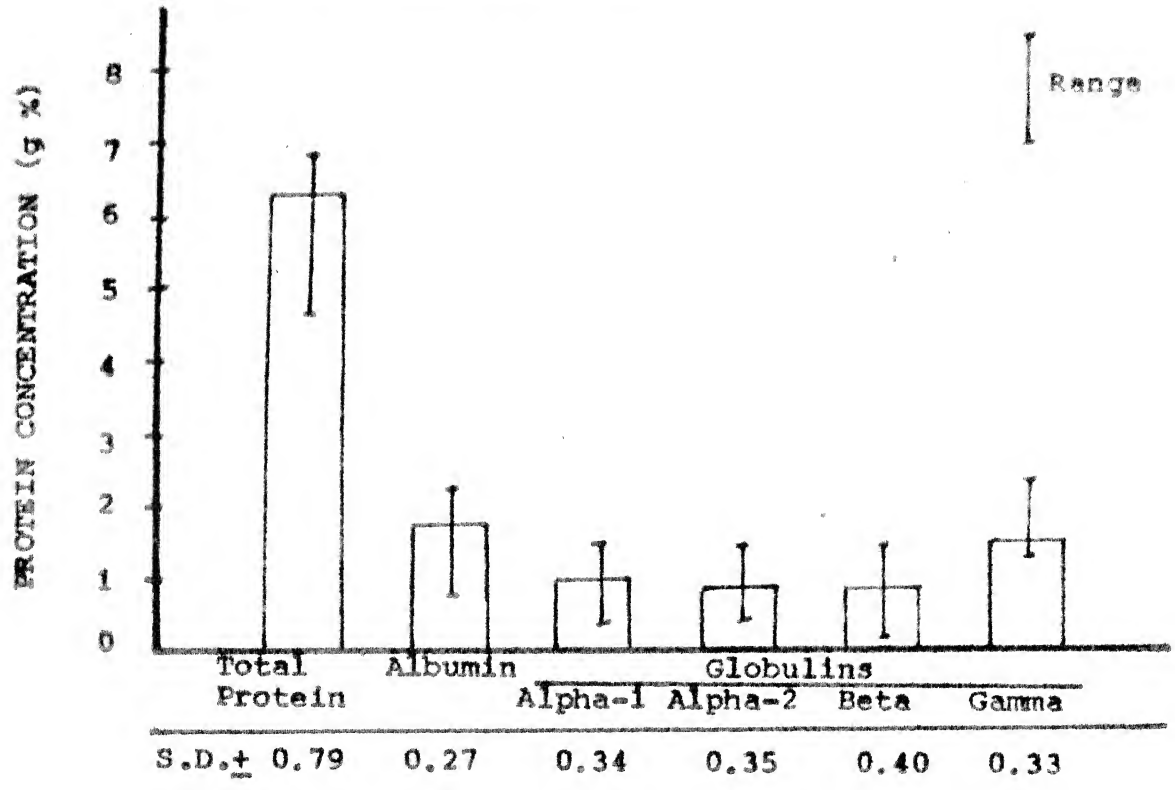
2. CIRRHOSIS LIVER




| | Total Proteins | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
|---|---|---|---|---|---|---|
| S.D.± | 0.94 | 0.55 | 0.32 | 0.36 | 0.53 | 0.63 |

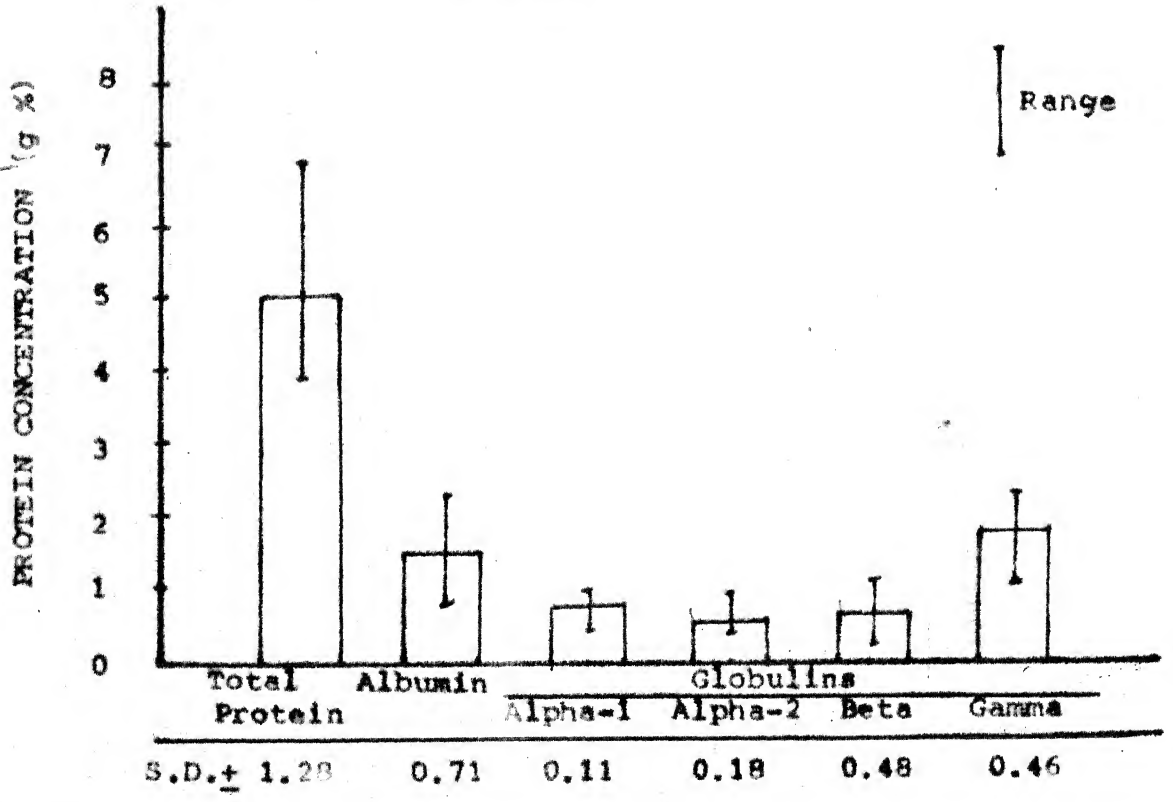
SERUM ELECTROPHORETIC FRACTIONATIONS

1. CHRONIC PERSISTENT HEPATITIS



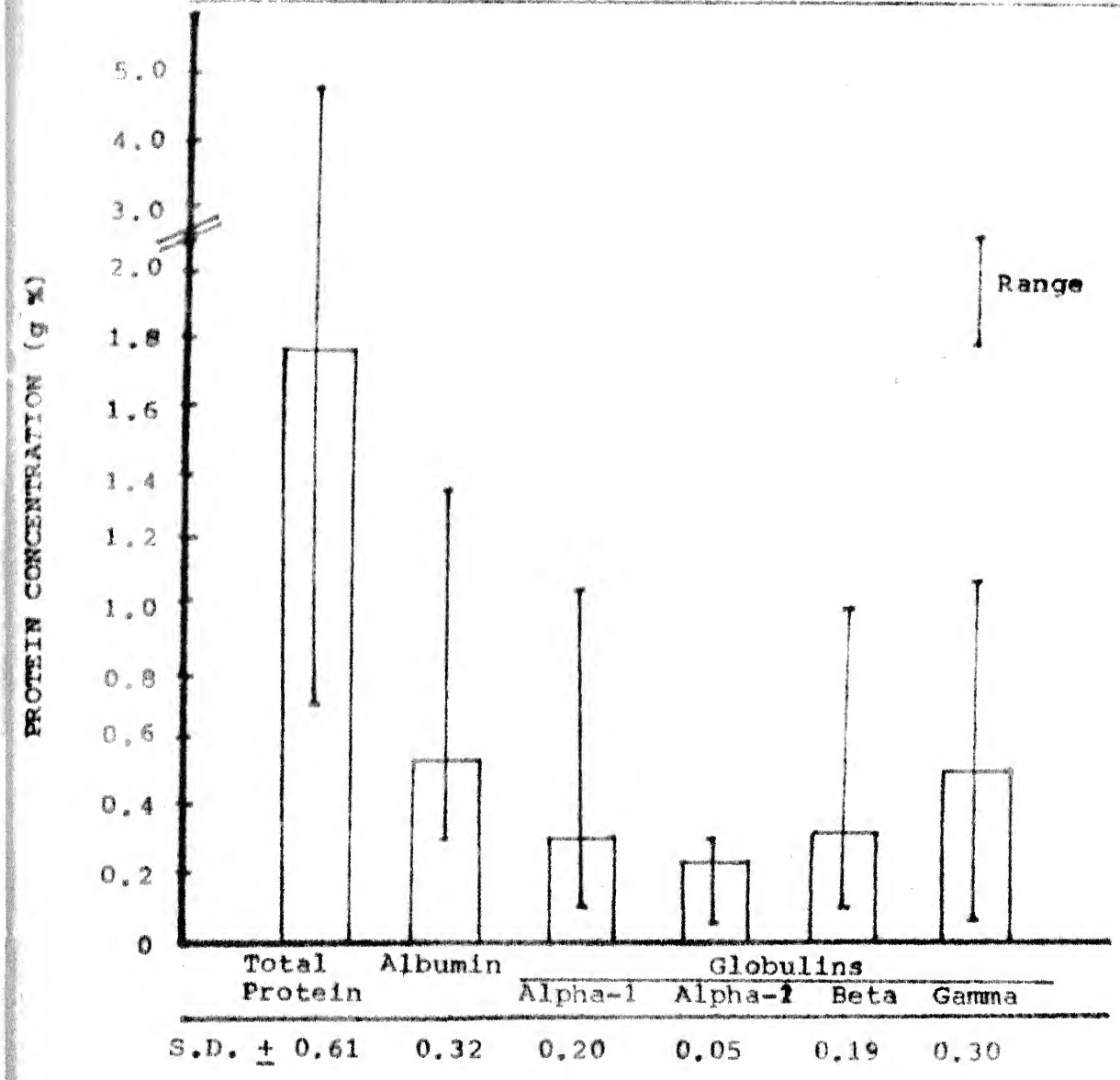

| | Total Protein | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
|---|---|---|---|---|---|---|
| S.D.± | 0.79 | 0.27 | 0.34 | 0.35 | 0.40 | 0.33 |

2. CHRONIC ACTIVE HEPATITIS

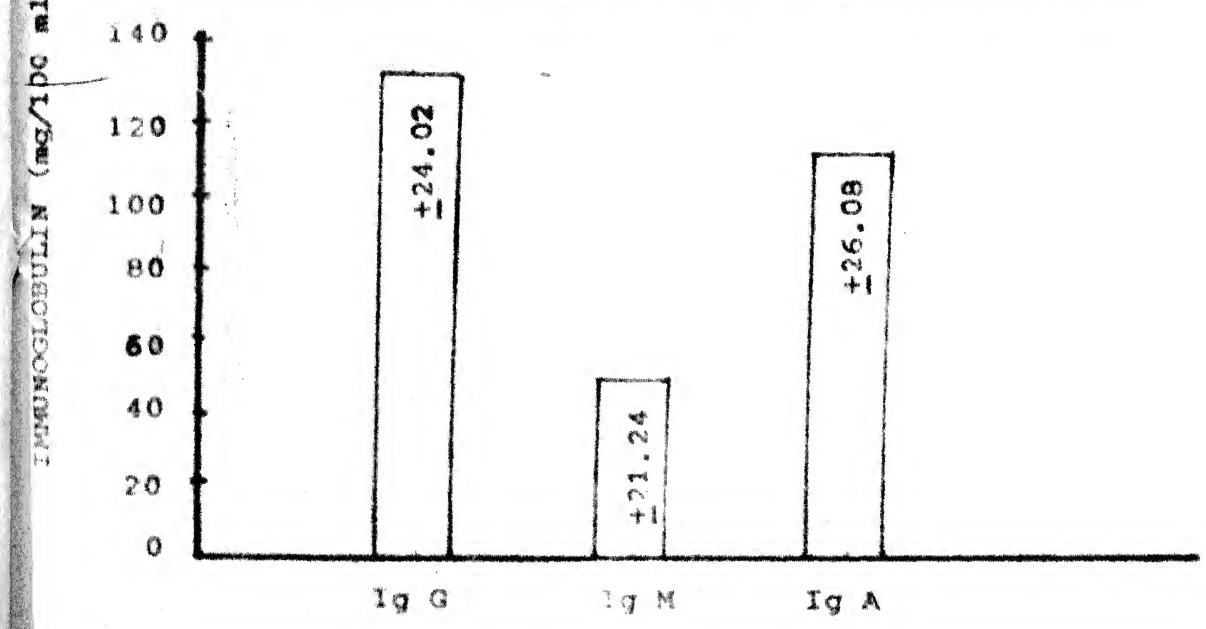



| | Total Protein | Albumin | Alpha-1 | Alpha-2 | Beta | Gamma |
|---|---|---|---|---|---|---|
| S.D.± | 1.28 | 0.71 | 0.11 | 0.18 | 0.48 | 0.46 |

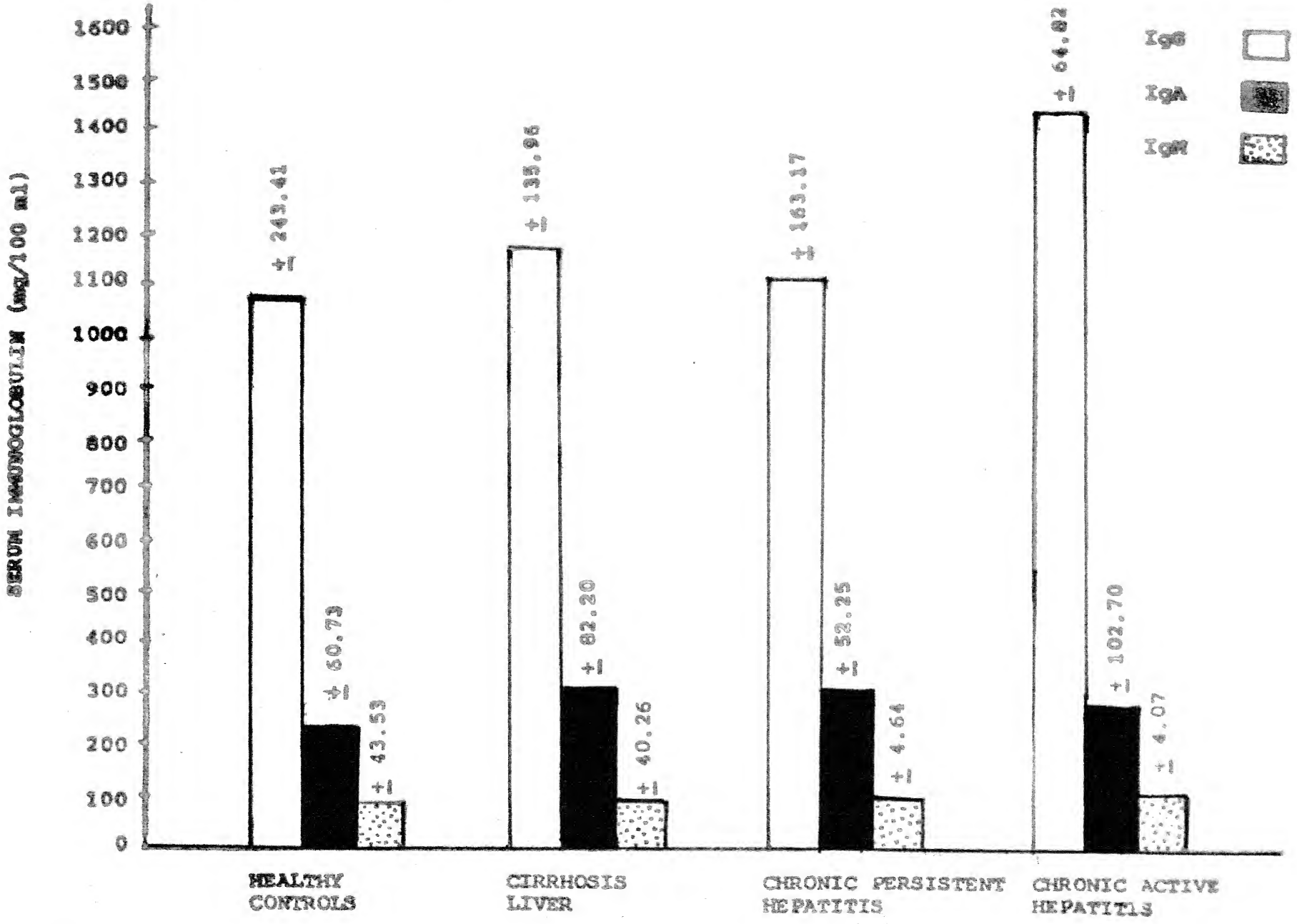
ELECTROPHORETIC FRACTIONATIONS OF ASCITIC FLUID PROTEIN



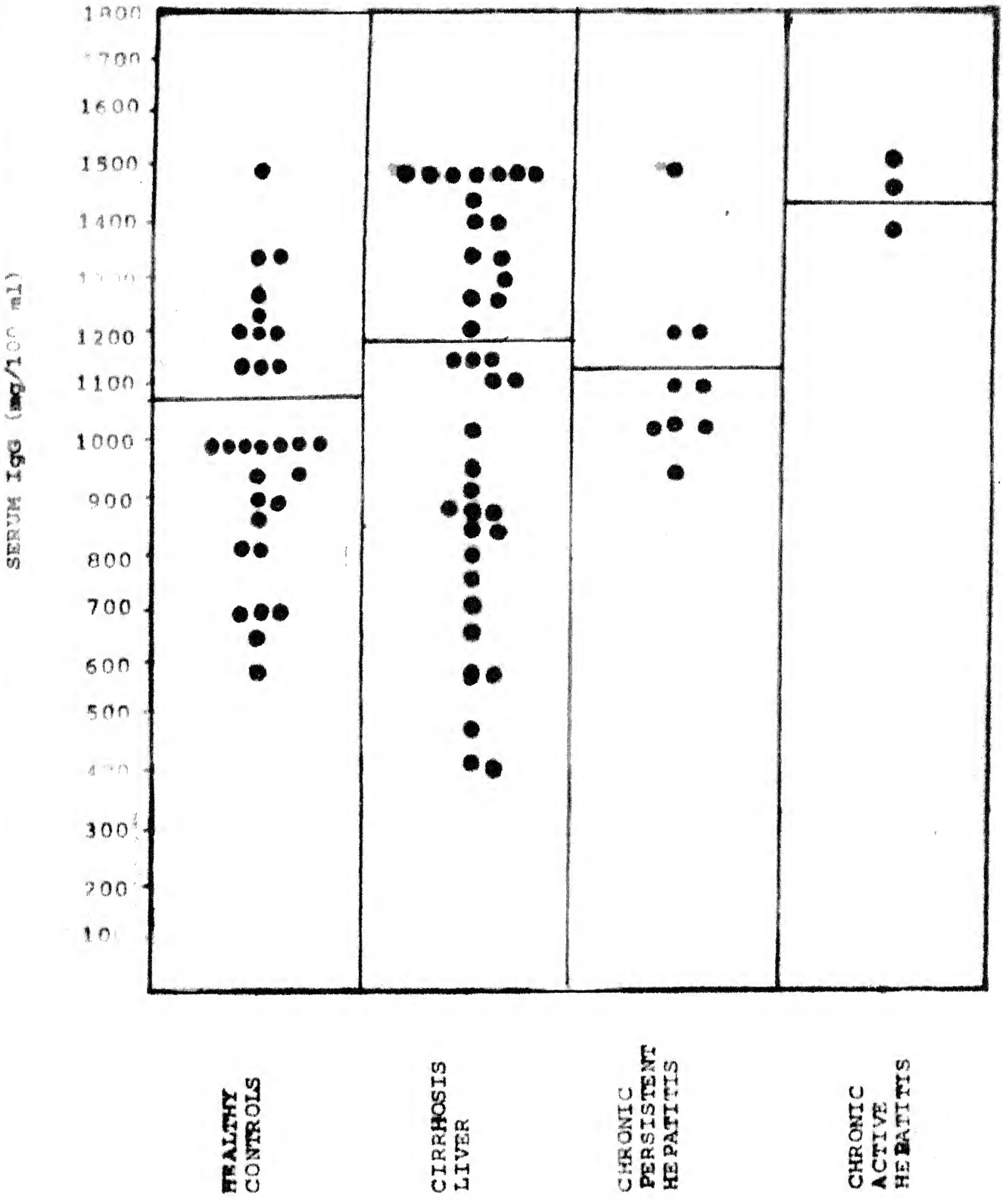

IMMUNOGLOBULINS IN ASCITIC FLUID (CIRRHOSIS LIVER)



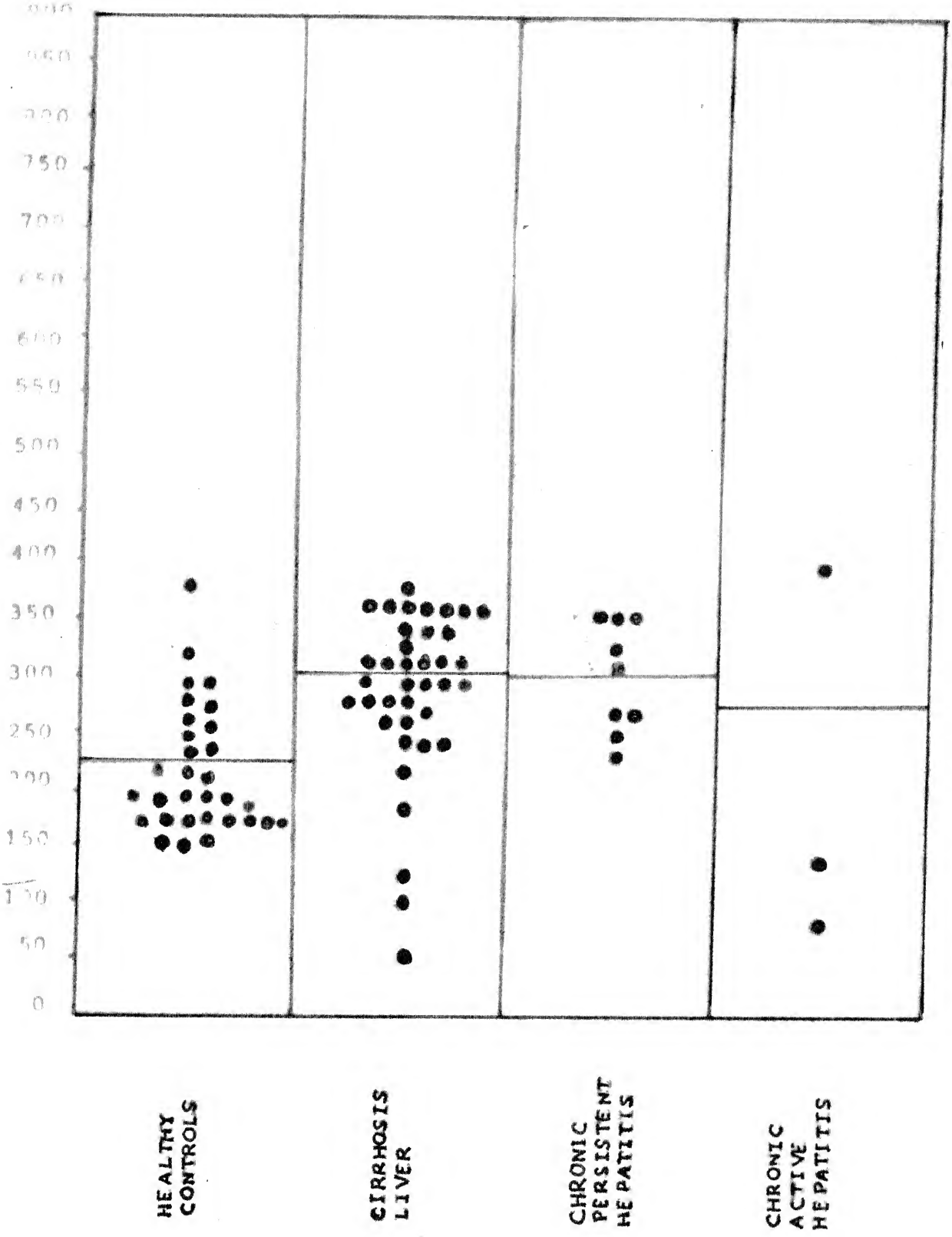
SERUM IMMUNOGLOBULINS IN CHRONIC LIVER DISEASES AND CONTROLS



SERUM IMMUNOGLOBULIN IgG IN CHRONIC LIVER DISEASES AND CONTROLS



SERUM IMMUNOGLOBULIN IgA IN CHRONIC LIVER DISEASES AND CONTROLS



SERUM IMMUNOGLOBULIN IgM IN CHRONIC LIVER DISEASES AND CONTROLS

# DISCUSSION

# D I S C U S S I O N

The liver is an active site of protein synthesis under normal conditions. The liver is responsible for the synthesis of the serum proteins, excepting the immunoglobulins (Gordon & Humphrey,1960; Martin & Neuberger, 1957 and Miller et al., 1954). In chronic inflammatory diseases of the liver, serum gamma globulins rise two or more times to normal. This increase is not due to a greater synthesis by liver cells but the littoral cells responding to the injury. The concurrent decrease of serum albumin is a measure of liver damage and reflects diminished synthesis (Cassarmal and Takatuski, 1953). The clinical application of electrophoretic fractionations of serum proteins in hepatic disorders was first established by Gray & Barron, 1943. Subsequently extensive studies were done on serum proteins by this technique (Brauto, G., 1951; Demoulenaere, 1961 and Martin, 1946). These workers recognised the characteristic patterns of serum proteins in hepatic disorders. Similarly the circumstances, primarily responsible for accumulation of ascites, remain speculative, despite the recognition of several

potentially relevant disorders of hydrostatic or osmotic equilibrium in hepatic diseases. Keeping this view in picture, electrophoresis of ascitic fluid was carried out to see any correlation of ascitic fluid protein changes with that of serum proteins alterations in hepatic disorders.

I. ELECTROPHORETIC PATTERN OF SERUM PROTEIN IN CIRRHOSIS LIVER.

In cirrhosis, significant diminution of the mean concentration of total serum proteins, serum albumin and alpha globulins and a rise of beta and gamma globulins are well known changes. These findings were reported for the first time by Gray & Barron (1943). Subsequently other workers also reported similar findings (Martin et al., 1941; Post & Patek, 1942; Wuhrmann & Wunderly, 1960; Cohen & Gorden, 1962 and Anderson, 1964). In the present study the total serum proteins were low in comparison with control group and diminution was significant ($P < 0.001$). Similarly significant decrease in the level of serum albumin ($P < 0.001$) was noted. The raised levels of alpha globulins were noted but the difference was insignificant

statistically (P >0.05). The beta globulin was not raised instead showed tendency to decline, although insignificant statistically (P >0.05). The gamma globulin level was above normal and the difference was significant (P <0.01). The decrease, though insignificant statistically in beta globulin, might be due to poor separation between beta and gamma globulins. The beta-gamma bridging i.e. lack of demarcation between beta and gamma globulins peaks described as a characteristic feature of the electrophoretic pattern in hepatic cirrhosis (Berg et al., 1961; Ceschka et al., 1958; Wuhrmann et al., 1960 and Zimmerman et al., 1957). Demeulenaere and Weime, 1961 and Tomasi et al., 1965 explained these findings on the basis of four fold increase in the concentration of immunoglobulin IgA as compared to only two fold increase in the concentration of immunoglobulin IgG.

II. IMMUNOGLOBULIN IN CIRRHOSIS LIVER

The hypergammaglobulinaemia as noted above in cirrhosis liver is likely to be a part of immunological response (Cohen, 1963). The antibodies against, the cell nuclei (Doniach, Roitt Walker and Sherlock, 1963) Mitochandria (Walker & Sherlock, 1965) Smooth muscle (Johnson Holbrow, 1965) are only few antibodies produced

in chronic liver disease. The raised levels of IgG and IgA were reported in cirrhosis liver by various workers (Maclachlan et al., 1965; Peizi, 1965 and Deicher et al., 1949). The very high level of IgA was reported in alcoholic cirrhosis by Mckelvey & Fahey, 1965 and Lee, 1965. In the present work the high levels of immunoglobulins IgG ($P < 0.05$) and IgA ($P < 0.001$) were noted. The immunoglobulin IgM level was decreased in comparison with control group. The difference in IgM level was not significant statistically ($P > 0.1$). These findings were similar as reported by other workers (Fahey & Sherlock, 1968).

III. ELECTROPHORETIC AND IMMUNOLOGICAL CHANGES IN ASCITIC FLUID.

The immunoelectrophoretic studies confirmed the presence of nearly all the plasma proteins in ascitic fluid, due to cirrhosis liver (Szabo et al., 1963; Schultz and Heremans, 1966). They also reported slightly increased concentration of albumin and alpha globulins fractions in ascitic fluid as compared to plasma. The beta and gamma globulins were also present but in lower concentration as compared to plasma. In the present study the albumin level was almost identical in serum and ascitic fluid i.e. 29.6% and 30.5% respectively. The beta and gamma globulins were in the

concentration of 14.5% and 28.8% in ascitic fluid as compared to 13.1% and 34.5% in serum respectively. This amply support the view that ascitic fluid contains all the fractions of plasma proteins in same proportions in dilute form.

All the three major immunoglobulins (IgG, IgM and IgA) were found in ascitic fluid. Chodirker and Tomasi (1963) reported that IgA and IgG ratio was always less than one in internal secretions such as pleural fluid ascitic fluid, amniotic fluid and synovial fluid. The IgA here is not of secretory type and the IgG and IgA ratio is similar to plasma that is approximately 5 : 1. In the present study all the three immunoglobulins were found in ascitic fluid. The IgG and IgA ratio was 1.4 : 1 in ascitic fluid as compared to 3.8 : 1 in plasma respectively. Thus slightly higher ratio of IgA was noted in ascitic fluid.

CHRONIC PERSISTENT HEPATITIS AND ELECTROPHORETIC FRACTIONS OF SERUM PROTEINS

In patients of chronic persistent hepatitis, the low concentration of total serum proteins and that of serum albumin were reported by Willcox and Bacher (1961) and Gelzayd & Kirsner (1967). The alpha proteins levels were not altered much, the beta and gamma

globulins levels were raised above normal in consistent with other chronic inflammatory disorders of liver, as reported by Martin (1946), Gutman (1948) and Ricketts (1949) & Sunderman (1968) also reported diminution of serum albumin, slightly raised level of beta globulin and raised level of gamma globulin. In the present work, the total serum proteins and serum albumin were significantly low ($P < 0.01$) and ($P < 0.001$) respectively. The alpha globulin concentration was slightly higher as compared to control subjects, but the difference was insignificant ($P > 0.5$). The beta globulin level was low but difference was insignificant ($P > 0.1$). The gamma globulin level was found to be raised. The difference, however, was insignificant ($P > 0.5$).

SERUM IMMUNOGLOBULINS IN CHRONIC PERSISTENT HEPATITIS

The serum immunoglobulins profile was altered in chronic persistent hepatitis. The raised IgG level was reported by Dienson et al.(1950). Fakuda et al (1978) reported that IgG and IgA levels were raised, while IgM level was within normal limits. The increase in all three class of immunoglobulins were reported by Lee (1965) and Feizi (1968). In present study the raised levels of IgG and IgA were noted but the difference

was statistically significant only in IgA class ($P < .05$). The immunoglobulin IgM level was almost identical with control group and the difference was insignificant ($P > 0.5$).

CHRONIC ACTIVE HEPATITIS AND ELECTROPHORETIC FRACTIONATIONS OF SERUM PROTEINS

The hypergammaglobulinaemia is the characteristic feature of this syndrome. Zimmerman and associates, who first discribed this syndrome in (1957), suggested that marked hypergammaglobulinaemia indicating increased production of anti-liver proteins and hypothesized that the syndrome represented a auto-destructive process. Bearn (1950) and Kunkel et al (1957) reported 26 instances of this syndrome with hypergammaglobulinaemia. The gamma globulin peak characteristically was a broad band which was attributed due to increase in all the immunoglobulins (Zimmerman et al, 1957). In the present work the total serum proteins and serum albumin were significantly low ( P values $< 0.001$ and $< 0.01$ respectively. The alpha globulins were low but the difference was insignificant. The beta globulin was also low but difference was not significant ($P > 0.1$). The gamma globulin level was raised but difference was not significant ($P > 0.5$). In this study the population of this

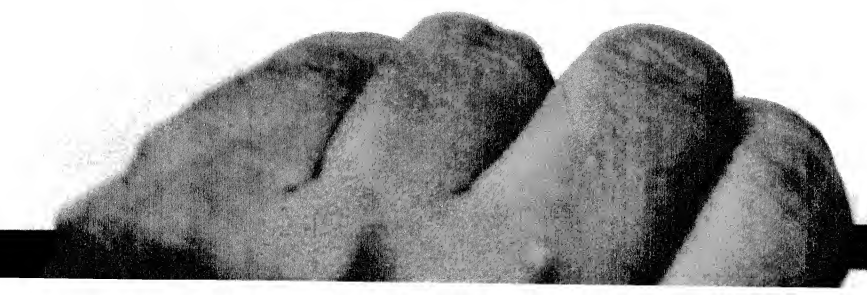
group was small, so nothing can be commented about the statistically insignificant rise of gamma globulin.

SERUM IMMUNOGLOBULINS IN CHRONIC ACTIVE HEPATITIS

Lee (1965), Mieschor (1966) and Deicher et al (1960) reported high levels of IgG and less striking increase in IgM and IgA levels in serum. While other workers like Machlachlan et al (1965) and Feisi (1968) reported no increase in the level of IgA while IgG and IgM were raised. An increase of IgM particularly after steroid therapy was reported by Wollheim (1957). An increased number of immunoglobulin producing cells were found in the presence of piecemeal necrosis and lymphoid infiltrates (Hadziyannis et al., 1969).

In the present study all the immunoglobulins (IgG, IgM and IgA) were found to be raised, but IgG level was significantly higher ($P < 0.01$). The difference in IgM and IgA level was insignificant ($P > 0.5$ and $> 0.1$ respectively).

# SUMMARY AND CONCLUSION



# SUMMARY AND CONCLUSIONS

The present study was carried out on 50 patients of chronic liver diseases. The study group consisted of 38 cases of cirrhosis liver, 9 of chronic persistent hepatitis and 3 cases of chronic active hepatitis. Thirty individuals served as controls. The total serum proteins and its electrophoretic fractions were estimated in healthy controls and study group. The immunoglobulins IgG, IgM and IgA were also estimated in both the groups. Similarly total ascitic fluid protein, its electrophoretic fractions and immunoglobulins were estimated in 26 cases of cirrhosis liver who had ascites. Following conclusion could be drawn from the study.

1. Mean total serum proteins level in healthy controls was 6.88±0.62 g%. The mean levels of various electrophoretic fractions were as follows, albumin, 2.86± 0.56 g%, alpha-1, 0.60±0.47 g%, alpha-2, 0.66±0.26 g%, beta, 1.05±0.55 g% and gamma globulins 1.71±0.50 g%.

2. Mean levels of immunoglobulins, IgG, IgM and IgA in control group were 1062 ± 243.41 mg%, 227 ± 60.73 mg% and 98.03 ± 43.53 mg% respectively.

3. Statistically significant decrease of total serum proteins ($P < 0.001$) and albumin ($P < 0.001$) levels

were found in patients of cirrhosis liver. The gamma-globulin level was significantly higher ($P > 0.01$).

4. The immunoglobulins IgG and IgA levels were raised significantly in cirrhosis group ($P < 0.05$) and $< 0.001$ respectively). The IgM level was low but insignificant statistically ($P > 0.1$).

5. The mean level of ascitic fluid protein was $1.78 \pm 0.61$ g%. After electrophoresis, the resultant fractions were compared with serum of patients of cirrhosis liver. Here all the protein fractions were present in ascitic fluid although in low concentrations. The lower proportion of gammaglobulin in ascitic fluid (28.8%) was noted as compared to serum (34.50%). The albumin and alpha globulin were present in almost similar ratio as in serum (Table IX).

6. All the three immunoglobulins IgG, IgA and IgM were present in ascitic fluid. The IgG was predominant immunoglobulin in ascitic fluid (47.3%). The IgA was next immunoglobulin in abundance (35.5%). The ratio of IgG and IgA in serum and ascitic fluid was 3.8 : 1 and 1.4 : 1 respectively. Thus raised level of IgA was recorded in ascitic fluid in this study.

7. In chronic persistent hepatitis group the significantly low levels of total serum proteins ($P < .01$) and albumin ($P < 0.001$) were recorded. The mean level of

gamma globulin was raised above normal but statistically insignificant ($P > 0.5$).

8. The raised levels of immunoglobulins IgG and IgA were recorded in cases of chronic persistent hepatitis but the difference was significant ($P < 0.05$) in IgA class only. The IgG level was low as compared to control group but difference was insignificant statistically ($P > 0.5$).

9. The significantly low levels of total serum proteins ($P < 0.001$) and albumin ($P < 0.01$) were noted in cases of chronic active hepatitis. The gamma globulin level was above normal but difference was not significant ($P > 0.5$).

10. The raised levels of all the three immunoglobulins were found in chronic active hepatitis group. The difference was significant in IgG class only ($P < 0.01$).

The chronic liver diseases can be characterised by low levels of total serum proteins and serum albumin. The gammaglobulin levels were found elevated in all the three groups studied. However, no distinct pattern of electrophoresis of serum proteins were found. The immunoglobulins IgG and IgA levels were found to be elevated in chronic liver diseases. The electrophoretic pattern

of ascitic fluid showed similar pattern as that found in serum proteins in cirrhosis of liver. The immunoglobulin IgA was found in higher proportion in ascitic fluid as compared to serum.

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## APPENDIX I

CLINICO IMMUNOLOGICAL PROFILE IN CHRONIC LIVER DISEASES

Date

Case No. M.R.D. No.

1. Name
2. Age/Sex
3. Occupation
4. Address
5. Date of admission
6. Physician Incharge
7. Ward/Bed No.
8. Clinical Diagnosis
9. Chief complaints
   1.
   2.
   3.
10. Short H/O of present illness
11. History of (a) drugs used
    (b) repeated injections
    (c) blood transfusion
12. Dietary History
13. General Examination

| | |
|---|---|
| 1. General condition | 8. Cyanosis |
| 2. Pulse | 9. Anaemia |
| 3. Blood Pressure | 10. Oedema |
| 4. Temperature | 11. Lymphnodes |
| 5. Hydration | 12. Skin |
| 6. Clubbing | 13. Palmer erythema |
| 7. Jaundice | 14. Alopecia |

15. Nails
16. Purpura
17. Testicular atrophy
18. Gynaecomastia
19. Any other

14. Systemic Examination

Abdomen

(a) Inspection
- shape
- visible veins
- flow of blood
- any lump

(b) Palpation
- signs of fluid
- liver
- spleen
- any other lump

(c) Percussion
- fluid thrill
- shifting dullness
- upper border of liver
- others

(d) Other systems

15. Investigations

(a) Blood Routine
- T.L.C.
- D.L.C.
- Hb %
- ESR

(b) Stool
- colour
- Ova
- cyst
- occult blood

(c) Urine
- bile salts
- bile pigments
- urobilinogen.

(d) Liver Function Tests - Vandenberg Reaction
- Serum bilirumin
- Thymol turbidity
- Serum Alkeline Phosphatase
- S.G.O.T. & S.G.P.T.
- Serum Cholesterol

16. Ascitic fluid

(a) - Colour
- Total proteins
- A/G ratio
- Cells

(b) Immunoglobulins in ascitic fluid

17. Total Serum Proteins and A/G Ratio.

18. Immunoglobulins pattern in serum

19. Liver Biopsy

SUMMARY